

Nuclear Fusion or the Sun God?

ONCE upon a time it was predicted that the population of the world would breed itself to death within a few years. Fortunately, or it might even be said predictably, this has come to nothing. Now, yet another series of predictions are being generated which will have equally apocalyptic consequences if they come to fruition. To put them in their simplest form it is that there is a shortage of energy and that there is no foreseeable way in which the world can meet its energy demands once the twenty-first century begins.

The wheel has certainly turned full circle. It is now presumably predicted that a gradual cold death will face the population of the world once the energy resources are exhausted. Only a short few years ago the concern being expressed was that the excess heat generated by more and more power stations, presumably straining to their utmost to meet the ever increasing demands of an ever sophisticated society, combined perhaps with aircraft flying in the stratosphere which could alter the radiation balance in the atmosphere, would ensure that the temperature would rise to such an extent that life would gradually become unbearable. It is no wonder that mere onlookers of the environmental scene are bemused.

But the true picture, of course, lies in between the two extremes as painted here. There is indeed an energy problem but it is far from being a crisis. It is now up to responsible governments to insure for the future by devoting reserves towards long-term research on energy problems. A series of three programmes entitled *The Energy Crunch* on BBC Television which came to an end this week has done a little to put the British viewer in the right frame of mind for the future, although it is to be hoped that viewers saw all three programmes the last of which went some way to redressing the unfair balance of the first two. The BBC must be commended for many of its science programmes for it has on occasion conspicuously failed to provide unbiased and logical argument on many issues. The recent series of programmes can also be criticized to some extent in this respect.

The repeated assertions that the safety of nuclear power plants is in doubt, without repetition of the impressive safety record of the British nuclear power industry, was one of the most insidious aspects of the programme. It must be considered to what extent the seemingly deliberate destruction of nuclear power as a solution to the energy problem was motivated by the *raison d'être* of the programme makers. How indeed could such a strong representation be made for solar power and geothermal power if fission reactors, both the present generation and the fast breeders, had been shown to be what they are, the workhorses of the nuclear power industry in the coming years?

It must also be asked why it was that much more emphasis was placed on solar energy rather than energy from fusion as the basis of man's hope for the future.

Fusion research is at an advanced stage and although it cannot be said that such reactors will prove to be the salvation of mankind—many years of research are needed before such categorical statements can even be contemplated—more money can safely be placed on the probability of fusion providing a safe, economical and regular supply of power than on any system designed to derive power from the sun.

But the makers of *The Energy Crunch* are correct in one respect and that is that speculative ideas for generating energy on a large scale deserve attention. Solar energy is only one of these possibilities the potential of which was reported by a combined NSF and NASA panel at the end of 1972. The panel reported that the total energy requirements of the United States in 1969 for example could have been supplied by the sunlight incident on 0.4 per cent of the country's land area. But what of coal? Is there not a possibility that power can be extracted from this source by combustion underground thus saving the cost of mining? Cannot nuclear power be used to electrolyse water in order to produce a supply of hydrogen which is a readily transportable source of energy? It is only to be hoped that the energy laboratories, energy departments and energy units which have mushroomed all over the world in what seems like recent months will not only devote their time and efforts to estimating, predicting or projecting the future of our existing energy resources but that the right people within these institutions will be given their heads, and the finance, to pursue futuristic ideas.

100 Years Ago



Winters and Summers

A FRIEND writes to me:—"From my observations of climate here (Belfast) I should say that I never saw a severe winter followed by a really fine summer. The severest winters I remember were those of 1854-5, and 1859-60. The summer of 1855 was very wet, and that of 1860 deplorable. The finest summers I remember were those of 1842, 1857, and 1868; in every case the preceding winter was very mild."

I would add to this, that the severe winters of 1865 and 1870 were not followed by remarkably fine summers. The harvest weather of 1866 was unusually bad.

Can any of your readers throw light on this subject from carefully kept registers?

JOSEPH JOHN MURPHY

Old Forge, Dunmurry, June 6

from *Nature*, 8, 182, July 3, 1873.



OLD WORLD

Whaling Commission still in Trouble

THE International Whaling Commission celebrates its quarter centenary this year. And trailing its past history of decisions taken too late or not taken at all, the commission is in London again to debate the future of the whale.

The scene is much like last year's. Friends of the Earth are picketing, the press is clamouring to be let in, the scientists are cautiously producing their advice, and the commissioners are there to ensure that whaling continues.

The proposal for the ten year moratorium on all whaling will be discussed again and rejected again, the commission will agree to make further efforts to get the whaling countries that are not members of the IWC (Brazil, Portugal, Spain, Chile and North Korea) to join, and more noises will be made about strengthening the commission's machinery (a permanent secretariat will almost certainly be established). At the end of the day some progress will have been made.

As the week began the scientific committee had still not completed its assessment of the current situation, nor produced its recommendations. But it is plain that the committee will come out against a total moratorium (so long as certain species are protected there is no harm in exploiting healthy stocks, it argues), but it will suggest that the fin whale be better protected.

The fin is the largest of the baleen whales that the commission members still hunt. Quotas set for it in the Antarctic and North Pacific for the 1972-73 season were 1,950 and 650 respectively, a catch that was exceeded in the North Pacific by 108, but not met in the Antarctic by 189. Stocks of the whale are heavily depleted, and it is calculated by the scientists that the species will have to be left unfished for 23 years for it to return to its maximum sustainable yield (MSY)—the maximum number that can be taken from any population without depleting the stocks.

At present the scientists estimate that 3,000 fins could be taken in the Antarctic without damaging the stocks further, but many more could be harvested if the fin is allowed to recover unhunted. It is therefore on the cards—although rather an outside possibility—that the conference will agree to a moratorium on the fin whale.

Dr Robert White, Director of the United States National Oceanic and Atmospheric Administration, opened the bid for a moratorium on all whaling on the first day of the meeting. He went

on to say that "placing the fin whale under a complete moratorium is the most urgent matter before this commission". Mr Michael Stodart, Minister of State for Agriculture, Fisheries and Foods, also put the weight of the British delegation behind the moratorium on the fin whale. Without actually calling for a ban in so many words, he said "if this

species is to recover to its maximum sustainable yield as fast as we would wish, the commission must be prepared to take much more stringent conservation measures. Indeed we believe that this is the species with the strongest case for a moratorium".

The scientific committee's views will not actually include a recommendation

POLLEN

Keep on Counting

IT'S been a good year for pollen and a bad one for hay fever so far. But, in spite of the estimated three million sneezing individuals spread across Britain, the pollen count has not been as high this year as it has in the past.

This year's record count to date—on June 19—rose to 351, the highest level for two years. But the largest figure in the records of the Asthma Research Council, which faithfully keeps the daily count from the roofs of Paddington, is a massive 820, recorded on June 17, 1964.

The pollen that causes all the trouble is grass pollen. Records are also kept of the amounts of tree, flower and nettle pollen in the atmosphere, but they are not published daily. Relatively few people are allergic to them. But some three million people are allergic to grass pollens—and nobody knows why. There are, literally, thousands of different allergies found, but grass pollen is the commonest. Of the unlucky three million, some 0.5 million need medical treatment. Once the particular pollen or pollens that are setting up the allergy have been identified (no easy task as there are hundreds of different grasses), a desensitizing vaccine can be given and the suffering eased. Which is of little comfort to the majority of those allergic, who snuffle away all summer whenever the pollen count creeps up around the 100 mark but who aren't ill enough to go to a doctor.

But although this year does not hold the record for a high pollen count, the levels found during the recent heatwave were unusually high, particularly for mid-June. Normally pollen is released from early June to August, and the count tends to rise steadily, peaking in July. This year, however, damp weather in May and early June held the release of pollen back, and when the heatwave arrived the pollen sacs in the

grasses were loaded, so that when the Sun finally burst them more pollen than usual was released at one time. One reason why so many people who normally do not suffer from hay fever were affected this year may be the speed at which the pollen count rose, defeating the body's immunity response which can build up resistance to the pollen if the quantities in the atmosphere accumulate slowly.

Nonetheless, the pollen count is highly elastic. Once the pollen is in the atmosphere it tends to stay there until it is flushed out by heavy rain. Thus the count was 351 on Tuesday 19, but only 3 on Thursday 21 following the heavy rain of last week. Friday's warm Sun had the count up to 69 again. Light showers will do little to shift the pollen in the air and will only encourage the grasses to produce more.

The count is taken with a spore machine that takes air in against a sticky microscope slide that revolves once every twenty-four hours. The pollen sticks to the slide and the grains are counted at noon each day, with the final count being the average number of grains found per cubic metre of air over twenty-four hours.

Although the Asthma Research Council's count is actually taken in London, it is held to be reasonably accurate for the whole of the home counties as pollen is so light that it can be blown miles once it has been released.

The Asthma Research Council counts the pollen largely because hay fever is a closely related disease to asthma. The council spends £41,000 a year on asthma research, including in its work studies on hay fever and other allergies. Research has revealed that the excreta from house dust mites are a chief cause of asthma; the researchers have also come up with the rather depressing conclusion that while many adolescents grow out of hay fever, a number of adults progress in middle age from hay fever to asthma.

for a moratorium. All the committee will do is outline what will happen if various courses of action are taken.

The best that can be hoped for is that the quota on fin whales will be further reduced, that firm quotas will be established for other species—minkies and sperm whales in particular, that a permanent secretariat, including a number of scientists, will be established, and that a more successful attempt will be made to bring the non-IWC members into the camp.

Some improvement in monitoring the number of whales caught during the season would also be welcome. Monitoring is at present the task of the International Bureau of Whaling Statistics. This year when it set the date for the end of the season, the rate of catch promptly doubled, so that 745 minkie whales over the 5,000 quota set in the Antarctic were taken.

It is in any case as well that a total moratorium on whaling will not be declared by the commission. The IWC has no hold over its members other than the hold of public opinion. Although Japan has been party to agreements to limit the numbers of certain species taken, she has still, for the past four or five years, taken whales outside her quota using a whaler sailing under a Brazilian flag (Brazil not being party to the IWC). The commission's constitution even allows for commission members to flout the commission's agreements, while still remaining members. If a total moratorium were to be imposed, it is more than likely that Japan and the USSR would ignore it, thereby destroying the IWC.

OECD

Research Efficiency

THE scientific standing of the smaller countries in Europe "is out of all proportion to their comparatively limited resources" according to the Organization for Economic Cooperation and Development. But several arguments suggest that "this very high grade research effort (cannot) be profitably continued, particularly in university laboratories, purely by following the main trends of international scientific activity as in the past".

This verdict emerges from the second of the OECD's expected three volume analysis of the research systems in the major European countries and North America. The countries involved in the present study are Belgium, Netherlands, Norway, Sweden and Switzerland. (*The Research System*, Volume 2, OECD, £2.38.)

The current volume is the successor to the report in which the research council system in Britain was compared with the funding organizations for science in France and West Germany.

The patterns of research in the two sets of countries are very different, and this is based, the report says, in the conduct of scientific and technological activities particularly in relation to economic activities. As the report points out, in the set of countries analysed in the present report "whatever may have been the constraints of international competition, research and development policies have pursued a steady path." This contrasts sharply with the progress of science in Britain, France and West Germany which has developed according to a "saw tooth pattern full of sudden changes and sharp breaks".

The report states that the "scientific area which the smaller countries occupy in the mass of knowledge produced throughout the world seems far greater than their 'economic space'." But the OECD examiners feel that the high grade research effort present in these countries cannot continue profitably, particularly in university laboratories. They quantify their argument by pointing out that:

- As the volume of world research continues to increase so will the number of branches of research. If these countries attempt to follow every line then "scientists in countries of limited resources will court failure by dispersing their efforts".

- Several fields of research, especially in physics, are particularly attractive to researchers but their economic value is dubious and the research tends to be expensive.

- Considerable capital expenditure is needed in many scientific sectors where research cannot make further progress without much capital and without setting up large teams.

- International technological competition will force nations to make choices and to specialize more narrowly in the industrial rather than in the pure research sector.

The OECD, in its own way, advises these countries that little progress can be made in the future without scientific effort being based on an overall strategy or, in other words, a national policy for research. Britain, of course, does not subscribe to this view and the OECD report does acknowledge that the need for countries to work out national research strategies is not always economically urgent.

Readjustment is the key word for these five countries, according to the report. None of the countries has assumed responsibility for large scale technological development — like the large industrialized nations—and so the difficulties of readjustment are not as large as they could be. Resources can be reallocated without large scale redundancies among scientists and engineers.

But the real challenge is yet to come.

The capacity of these countries for readjustment in the industrial fields has already been shown, but will they succeed in harnessing this capacity with the same energy in the solution of their major socio-economic problems? The crucial question, according to the report, is whether "they will be able to equip themselves with institutions devoted to scientific research which are capable of making the most rigorous and essential choices and yet avoid paralysing unofficial initiative with red tape." The OECD, this time, has hit the question right on the head.

ROYAL SOCIETY

Financial Problems

THE Royal Society is to double its fellowship fees. At a recent meeting of the fellowship it was agreed that the annual subscription fee will go up from £10 to £20. This decision has still to be ratified by July 12's council meeting and the society's statutes will have to be changed before it comes into effect.

The reason for the rise is simply impecuniness. In spite of the large grants that the Royal Society handles from Parliament, the society's general purposes fund, which is used to run the society's own business, has fallen into deficit. The account was £100,000 down last year, double the deficit in 1971.

The situation is being aggravated by the fact that many of the covenants that the Royal Society received in 1966 and 1967 just before it moved to Carlton House Terrace are now running out. These covenants produce an income of £50,000 a year. The society has also been hit by the £30,000 loss made on its publications last year, the first loss recorded for 18 years.

Sir David Martin, the Royal Society's secretary, said this week that doubling the subscription "will only be a small help". The society only has just over 850 fellows, so that an increase of £10 a head will only raise something under £9,000. But, says Sir David, "by beginning with an increase in the fellows' subscriptions, we are at least showing that we are willing to help ourselves".

Like many other scientific bodies — the Royal Institution for example, which has largely weathered its immediate financial problems (see *Nature*, 243, 3; 1973)—the Royal Society has been hit by inflation. The society has always tried to keep its publications work at just better than break-even, with price increases infrequent. The cost of some of the society's publications has already gone up this year—the *Proceedings* for example—and the society is clearly going to have to make a little more profit in future from publications in order to help make ends meet. A further appeal to help redress the general purpose fund's deficit is also likely.

NEW WORLD

Scientific Agreements Signed in the United States

from our Soviet Correspondent

THE agreement signed last week between the United States and the Soviet Union on cooperation in agriculture, transportation, ocean studies and cultural and scientific exchange, together with the agreement on nuclear arms limitation and the peaceful uses of atomic energy would seem, at first sight, to be a major step forward in scientific and technical cooperation between the two powers.

But a great deal of the ground covered is not so much an innovation as a ratification in greater detail of the Soviet-United States agreement of May 24, 1972, which left room for further cooperation between the countries.

Cooperation in oceanography has already begun with such projects as the two month long meteorological survey of the Bering Sea (*Nature*, **241**, 420; 1973), or is well into the planning stage as in the case of the joint Glomar Challenger mission (*Nature*, **242**, 289; 1973). A preliminary cooperation programme in marine pollution has already been drafted and the working group dealing with this problem first met in Washington at the end of May.

The new United States-USSR Environmental Commission, under the co-chairmanship of Academician Fedorov of the USSR and Dr Russell Train, Chairman of the United States Council of Environmental Quality, has already met some ten times this year.

Dr Lee Talbot, a senior adviser to Dr Train, said in London this week that the joint commission "has been a success" and added that the new cooperative programme is welcome.

Soviet reaction to the recent scientific agreements takes a double course. For external consumption, the *Novosti* press releases stress the benefit the United States may be expected to gain from the new arrangement. But *Pravda* stresses the advantages to be gained by the Soviet Union.

Thus whereas *Novosti* stresses that Soviet experience in collectivisation might "well prove to be useful" to the United States agricultural industry, an article on town planning (*Pravda*, June 16, 1973) in anticipation of the expected agreements, indicated that cooperation with the United States over "city environment" would play a considerable part in future Soviet planning.

Regarding future developments the agreement on scientific exchange is at

first sight ludicrously small, an annual interchange of "40 graduate students, young researchers and instructors for study and postgraduate research in the natural sciences, technical sciences, humanities and social sciences . . . at least 30 language teachers to participate in summer courses of ten weeks . . . at least 10 professors and instructors of universities and other institutions of higher learning to conduct scholarly research for periods of between three and six months" is all that is specified. But these are minimum figures, and presumably do not include participants in specific programmes otherwise covered. A separate clause also provides for short-term exchanges of academics.

But it may be noted that the British-Soviet exchange arrangements of January 1971 failed to materialize on the scale originally envisaged (see *Nature*, **229**, 482; 1972) so that those visits for prolonged research which did occur became matters for special comment rather than the routine of a working agreement.

The implementation of one clause in the ocean studies agreement could result

in acceptance by the Soviet Union of the 10-year moratorium on whaling that is currently under discussion at the International Whaling Conference in London. It is too early to forecast the outcome of the conference, but the large Soviet capital investment in pelagic whaling equipment makes acceptance of the moratorium unlikely. It remains to be seen whether this will be the first clause of the agreement to be broken.

The Nixon-Brezhnev talks have ended in an atmosphere of goodwill, with many references to cooperation already achieved with the progress already made in planning the Soyuz-Apollo project being inevitably cited. In the fields where the interests of both parties coincide, such as the project on oncological and vasculo-cardiac research for example, or where they are not in conflict (general environmental studies) good progress is already being made. The new agreements serve largely to approve and extend these promising beginnings. In cases where interests conflict, however, as in the current whaling issue, the outcome seems far less clear.

LARGE SPACE TELESCOPE

Experimental Definition

by our Cosmology Correspondent

NASA plans to launch a large space telescope (LST) by the shuttle sometime in the 1980s. The telescope will have a 3 m mirror, and may be up to 16 m long and 4 m wide, with a total weight of some 9,000 to 11,000 kg; this makes it one of the most important scientific experiments planned for the 1980s and representatives of four countries have now been chosen to define the experiments which will be carried out.

The LST will be able to examine galaxies 100 times fainter than those seen by the most powerful Earth based optical telescopes, but it is not intended solely to study the remote depths of space. Present immediate plans are for twenty-eight members of the experiment definition team to study proposals covering five areas of astronomy:

- high resolution spectrography
- low resolution spectrography
- imaging optics
- infrared devices
- astrometry

Data handling and operations problems for all experiments are being co-ordinated by a separate team. There are also five "at large" members of a scientific working group to oversee the development of the project: Lyman Spitzer, Princeton; Arthur Code, Wisconsin; Margaret Burbidge, RGO; John Bahcall, Institute for Advanced Study; and B. Field, Smithsonian Astrophysical Observatory. There can be no doubt that the glamorous astronomical subjects — the energy processes in galactic centres, supernova remnants, QSOs for example—will receive considerable attention. Conventional astronomy will not be ignored, however, and the LST will, for example, provide a good platform for long-term monitoring of atmospheric phenomena on Venus, Mars, Jupiter and Saturn.

A few details of the telescope and its systems have already been decided, as well as the basic size. The guidance system will be accurate to 0.005 arc s ("equivalent", according to NASA, "to locking onto a single strand of hair at a distance of two miles"), and the orbit will be at an inclination of 28.5°, reaching an altitude of 648 to 778 kilometres.

SOVIET JEWS

A Second Letter to an Imaginary Soviet Scientist

DEAR Colleague—Your kind invitation to attend the International Conference in Moscow this summer arrived this morning, and I hasten to reply. The programme is unusually interesting. Yet I am not quite sure whether I shall, in fact, accept the invitation. Something troubles me, and I need your advice as an old friend and colleague.

Let me say, first of all, that I have long wished to visit the Soviet Union, not only for its tourist attractions but in order to make closer contact with Soviet scientists in our field. In an earlier letter (*Nature*, **217**, 123; 1968) I remarked on some of the confusions that arise when arrangements are made for Soviet scientists to attend conferences in other countries. If you cannot easily come to us, then we should make special efforts to attend conferences and visit laboratories in the Soviet Union so as to keep each other well informed on our scientific progress.

In particular, I was looking forward to a long visit to your theoretical group, whose head has done such brilliant and exciting work over the last ten years. Imagine my distress, then, to see his name in the newspaper, and to receive from him, by a roundabout route, a heart-rending plea for help and support in terrible circumstances. You know much more about it than I do, for it must have hit you particularly hard that your young colleague, with whom you have worked so closely over so many years, should be dismissed from his post, and from all scientific work, for daring merely to ask that he might be allowed to emigrate to Israel. You and I as scientists, can feel the additional cruelty of the order that his books—the fruits of half a lifetime of effort—should be withdrawn from the libraries and that his papers should not be cited in future.

I understand perfectly that there is very little that you and your colleagues can do in the face of such forces. No doubt you are helping our friend in private, with the gifts and sympathy so characteristic of Soviet intellectuals. I suspect that you will also devise ways by which he can continue to do research in private and will be sure to keep his name in the scientific literature: no scientist who is remotely capable of judging the relevance of a citation would be an accomplice to this utter perversion of the system on which we all depend for our scholarly recognition.

If Soviet scientists are to be denied the

fruits of their honest labours, then Soviet science will soon be at the mercy of dishonest timeservers. You must be trembling inwardly with the thought that a higher patriotism than complete obedience to these orders would be to preserve for the Soviet Union the sceptical, critical, imaginative scientific way of life.

But my real question to you is—what should we do: what should I do, to help the scientific friend whom I have never met? I do not think I am greatly influenced in this by my own Jewish birth and upbringing, which in a free society I need neither exalt nor repudiate. A man in trouble has called on me by name, invoking the universalism of science, and the fellowship of our Invisible College, which knows no natural frontiers: I must surely heed his call.

One form of help is very easy to get in this case—publicity. All normal procedures of protest are being actively pursued, in the press, in government circles, through embassies, and so on. It is scarcely to be believed that much more can now be accomplished in this way.

Can anything be done through our scientific organizations and learned societies? It is my belief that this particular case—like a number of other cases in various countries (e.g. in recent years, Argentina and Czechoslovakia)—constitutes such an infringement of the norms of the scientific community that it is our duty to take notice of it officially. It seems to me, for example, that the agreements made between the Royal Society and the Soviet Academy of Sciences for scientific exchanges are based upon the assumption that these bodies have a similar respect for scholarly integrity, for scientific authority, and for the acknowledgment of scientific originality and priority. It would not, I believe, be a transgression of its principle of avoiding “politics” if the Royal Society were to question the official attitude of the Soviet Academy to these matters, and to indicate its unwillingness to cooperate with an organization that so openly failed to live up to the high ideals that we all profess to hold.

In the end, however, only a personal gesture is likely to be possible. That is why I am troubled about whether or not to accept your invitation to the Moscow conference. Should I refuse to take part myself in an activity sponsored by a government whose policy I detest, under

the auspices of an organization—the Soviet Academy—whose hypocrisy I deplore? I realize that such action is largely symbolic. At some cost to myself (for it really would be pleasant and scientifically profitable to come) I cause a little damage to those whom I wish to influence and fail in my friendly duty to people, like yourself, whom I like and trust. Yet I think that the gesture is not quite useless, and that if it were copied by the many hundreds of British, European and American scientists who have been invited to scientific conferences in the Soviet Union this summer it might bring out very clearly the extent to which, as a community, we are united in detestation of these present actions by your government.

Tell me, then, colleague and friend, what I should do. I have never agreed with those who demand a boycott on visits to this or that country—Spain, Greece, South Africa, Czechoslovakia—on general political grounds. A scientific visit may look like support for the policy of the government in power: but in reality, it may be valuable moral backing for those men of integrity and good will who are struggling to establish scientific standards under very adverse circumstances. I prefer to put my trust in such men, and to accept their personal invitations in the name of scientific fellowship.

Is this what you had in mind when you sent me this invitation? Would I come as an official guest of your government, expected to speak in admiration of your scientific achievements and never to express, even in private, my inner feelings on this tragedy? Or do you ask me as a personal scientific friend, not only to discuss our common interest in certain technical questions of scientific research, but also to share our common concern for what is being done to another friend by this cruel world in which we all have to live? I am not speaking, of course, of reckless public acts of defiance, or any other such extravagant, useless gestures: merely of the spirit with which you would yourself meet me on this occasion. Until I know that, allow me to reserve my reply. I hope that you will still want me to come; but I shall understand, and admire, a message to the contrary.

Yours sincerely,

JOHN ZIMAN

*H. H. Wills Physics Laboratory,
University of Bristol*

NEWS AND VIEWS

Solar Eclipse of a Lifetime, June 30, 1973

EVEN in the space age a total solar eclipse is a valuable tool in astrophysical and geophysical research. The longer the eclipse the more useful it is, and to attract ground-based scientists the Moon's shadow must cross some readily accessible land from which there must be a good chance of good visibility.

In the main, the solar eclipse tomorrow meets all these criteria. It is a very long eclipse with a peak totality of 7 min 4 s, which is not much less than the upper limit of 7.5 min, and which is considerably greater than the mean peak duration of 3 to 4 min. Not for several hundred eclipses, until June 25, 2150, will the duration of the forthcoming eclipse be exceeded. Peak totality will occur near where Niger, Mali and Algeria meet, which is not the most accessible of regions but, as the map shows, the shadow crosses more accessible countries, especially Mauritania and Kenya, where totality will last for not much less than the peak time. The chance of cloud cover is less than 30% over most of the African track, though deep in the Sahara atmospheric dust is likely to be a problem.

It is thus not surprising that the largest numbers of ground-based scientists will be in Mauritania and Kenya. Most of the French and Russians will be at Atar in Mauritania. Part of the US National Science Foundation (NSF) expedition will be at Chinguetti, Mauritania, and the rest of it will be at Loiyengalani, Kenya, except for a small group farther north at Koobi Fora at the edge of totality. At or near these principal sites there will also be several small groups from other countries, though a substantial number of Japanese will be at Eli Springs, on the opposite shore of Lake Rudolf from Loiyengalani.

Elsewhere, near the Cape Verde Islands, a Russian ship equipped with sounding rockets will be used as an observation platform, while just off the coast of Mauritania there will be a boatload of amateur astronomers from Britain. A few hardy groups will be strewn along the rest of the track, with a small concentration in Niger.

From the north of Mauritania an Aerobee rocket prepared by an Anglo-American group will be fired into the shadow, while coming in from the Canary islands will be Concorde 001 carrying two British experiments, two French experiments and an American one. Concorde 001 will fly along the eclipse path from Mauritania to Chad, thus extending totality to just under 80 min. Over the central part of this flight Concorde will be underflown by a group of American scientists in a USAF NC-135 aircraft, which will provide their ten or so experiments with 12 min of totality.

During a total solar eclipse it becomes possible to make Earth-based measurements that are otherwise difficult or impossible. Most of those planned are concerned with the solar atmosphere. This will be studied from the chromosphere, 1,500 km above the Sun's surface, out through the K corona (free electron scattering) to the F corona (dust scattering). There follows an indication of the range of solar measurements to be made on this atmosphere. No attempt has been made to be exhaustive. Finally, some of the experiments not associated with the solar atmosphere will be described.

The chromosphere is to have its optical spectrum recorded by a group from the University of Utrecht, and a group from Pennsylvania State University will study it at 3 mm and 8 mm to determine its temperature and density. Spicules in the chromosphere will be studied by a group from the High Altitude Observatory, Colorado. On board the Concorde a group from Queen Mary College, London, will examine the structure of the chromosphere by means of spectra obtained every 2 s between 0.3 mm and 1.5 mm. This experiment makes use of the 95% atmospheric transparency at these wavelengths at Concorde's 17,000 m altitude, and the slowed down motion of the Moon will result in improved height resolution of the chromosphere structure.

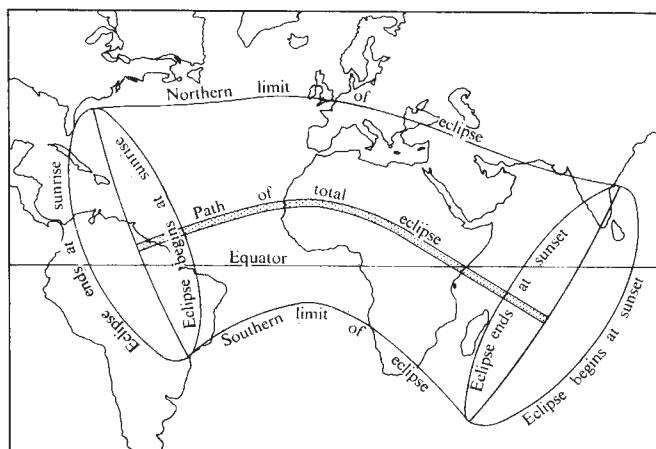
The chromosphere-corona transition is to be examined by the American Concorde team, from Los Alamos Scientific Laboratory (LASL). They will also look for waves in the lower corona, predicted to have a period of 300 s and which may "drive" the solar wind. The artificially extended totality will, of course, be a great advantage. A search for these waves will be made also by one of the French Concorde groups, from the Paris Astrophysics Institute, using a different technique.

The corona is the object of study of many of the experiments on board the NC-135. These originate from LASL and will involve spectroscopic, photometric and polarization studies at optical and near-infrared wavelengths. There are numerous ground-based corona experiments among which is a joint Franco-Russian effort to determine the time dependence of coronal jets by sequential observation from Mauritania and then Chad. This study is helped by the proximity of a sunspot minimum, which is associated with enhanced jet visibility. Various American groups will study coronal rotation, internal motion and solar latitude variations. A group from Sacramento Peak Observatory will try to detect certain broad ultraviolet absorption lines that have never been conclusively established.

The only ground-based rocket experiment has been prepared by a group from the Science Research Council at Culham and Imperial College, with rocket and support provided by Kitt Peak Observatory. The Aerobee rocket will reach a height of 150 km in the shadow and investigate the corona between 850 Å and 3,150 Å. This will increase knowledge of the structure, heating and magnetic activity of the corona and of any solar prominences that may occur. The solar prominences, like the weather, are capricious, but several ground-based groups also hope to investigate them.

The K corona gives way to the F corona at greater distances from the Sun. The K-F division is the subject of an experiment on the NC-135, and the F corona itself is to be studied by the other French Concorde group, from the Paris Observatory at Meudon, and also by various ground-based groups—for example a Franco-Russian group, and one from the University of Manchester which will be in Niger. The chief purpose of these experiments is to determine the spatial distribution of the F corona and composition of the dust grains comprising it.

There are a few experiments not concerned with the



Visibility of the total solar eclipse of June 30, 1973.

solar atmosphere. A group from the University of Texas will photograph the near-Sun star field to see if there are any distortions such as would be produced by gravitational bending of light grazing the Sun. A photographic search for new planets and comets near the Sun is to be made by a group from Dowling College. A few groups will be using the eclipse to make accurate determinations of the lunar diameter and orbit.

Finally, there are a small number of experiments associated with the Earth's atmosphere. The other British Concorde experiment will be conducted by a group from the University of Aberdeen who will observe at different heights in the Earth's atmosphere the decay of a metastable state of the oxygen molecule, normally sustained by solar radiation. An experiment on the NC-135 will examine forbidden emission lines arising from oxygen and nitrogen atoms in the upper atmosphere, while sounding rockets from the Soviet ship near the Cape Verde Islands will ascend to 180 km during the eclipse to record atmospheric density, ion composition, electron concentration and particle fluxes. On the ground a joint University of Botswana/University of Nairobi experiment will study the airglow, and a group from the University of Alaska will measure sky brightness. The relation between near ground atmospheric electricity and turbulence will be investigated by a group from the Naval Research Laboratory, Washington DC. A group from the University of Florida will investigate mechanisms of direct atmospheric solar heating by recording low frequency pressure waves, and a complementary group in Kenya from the Open University and Cornell University will carry out similar measurements.

This is clearly going to be an intensively studied eclipse. For those on the ground one must hope for clear skies.

B. W. J.

Dubious Mantle Plume

In the beginning was the Hawaiian chain, and the Hawaiian chain was the hotspot. The same was in the beginning with mantle plume. All things were made by mantle plume, and without mantle plume was not anything made that was made. Or so some earth scientists would appear to have us believe. But is the mantle plume the

true light or does it, as R. T. Chamberlain said of Wegener's original continental drift hypothesis, take "considerable liberty with our globe, [being] less bound by restrictions or tied down by awkward, ugly facts than most of its rival theories"? Though far from being resolved, this is an important question because if hot spots and mantle plumes are proved to exist, they may, as Vogt is to suggest in a forthcoming article, lead to a unifying hypothesis for the earth sciences.

In the meantime, the process of sifting the evidence goes on, and papers supporting the idea of hotspots and plumes are becoming noticeably more frequent. Moreover, general theorizing is now giving way to more rigorous analyses of particular plumes. In recent months, for example, there have appeared three particularly convincing reports in which different types of data have been used to reach remarkable agreement on the areal influence of the supposed Icelandic plume. Thus Schilling (*Nature*, **242**, 565; 1973) has used rare earth and minor element geochemistry, Francis (*Earth planet. Sci. Lett.*, **18**, 119; 1973) has used seismicity, and Vogt and Johnson (*Earth planet. Sci. Lett.*, **18**, 49; 1973) have used both morphology and seismicity to show that there is a distinct limit some 900 km to the southwest of Iceland beyond which the normal processes of seafloor spreading operate but within which the situation seems to be more complex.

On the other hand, those workers who have sought to use age data to plot plume traces across the lithosphere have been less convincing, partly because adequate sets of age data do not exist and partly, it must be said, because some ages are less consistent with the plume hypothesis than they might be. The only exception to this seems to be Clague and Dalrymple (*Earth planet. Sci. Lett.*, **17**, 411; 1973) who, notwithstanding serious gaps in the age data, are well on the way to making a good case for the applicability of the plum-hotspot hypothesis to the Hawaiian-Emperor chain. This is, of course, the region where it all started and where, some people would suggest, it ought to stay.

But in spite of the defects in the data, there is clearly something going on—and nowhere more so than in the vicinity of Iceland where conditions seem to be quite different from those elsewhere along the mid-Atlantic ridge system. Whether or not the differences are due to the effects of a rising mantle plume beneath Iceland is, of course, another matter; opinions are divided and perhaps barely formed. There has been no shortage of vociferous opposition to the idea of mantle plumes, although most of it has been limited to conferences and university common rooms, and few opponents have been willing to commit their reservations to writing. In view of the sense of embarrassment still felt by many earth scientists (including those born long after the event) at the way that Wegener and his early supporters were vilified in the press by much of the geological establishment of the day, this wariness may not be surprising. It would be quite understandable if the memory of this unfortunate period of history were to induce a reluctance to commit to print opposition to an idea which could well turn out to sweep the board and thus subsequently make its opponents look foolish.

On the other hand, it would be unfortunate, for it is highly desirable that alternative explanations of the data should be brought forward and thoroughly tested. The article by O'Hara on page 507 of this issue of *Nature* is

thus particularly welcome, for by seeking to account for the Icelandic condition without recourse to mantle plumes it represents one of the first serious attempts to show that data which have previously been taken to support plumes may well be explicable in more conventional terms. Thus O'Hara argues the case, rejected by Schilling, that the geochemical variations radiating from Iceland are caused not by the mixing of two primary magmas but by high and low pressure fractional crystallization dependent upon elevation. Presumably, the increasing elevation towards Iceland reflects greater magma flow but, as O'Hara admits, even his explanation begs the question of why this should be so. The point is, however, that if O'Hara's arguments are valid, the number of phenomena for which it is necessary to invoke a mantle plume is much reduced. P. J. S.

PROTEINS

Active by Association

from our Molecular Biology Correspondent
SHERLOCK HOLMES'S maxim that when you have eliminated the impossible, whatever remains, however improbable, must be the truth, has proved itself capable of wide application. It is implicit in the reasoning of Biswas and Paulus (*J. biol. Chem.*, **248**, 2894; 1973), whose studies on the subunit enzyme, aspartokinase, have brought to light a curious and so far unique situation. It is in all regards one of the more nightmarish allosteric systems, with control of activity by ligands operating apparently at three different sites, and, in addition, critical dependence of activity patterns on concentration and temperature.

The new work treats of the dependence of the functional properties on the subunit interaction. In the first place the stoichiometry has been established: in SDS-gel electrophoresis there are two subunits, α and β , with molecular weights of some 43,000 and 17,000, in equal molar proportions. The molecular weight of the native enzyme then corresponds to an $\alpha_2\beta_2$ structure. This Biswas and Paulus have confirmed by the invaluable cross-linking technique of Davies and Stark: treatment with a bifunctional imidoester leads to the appearance on SDS gels of four new components, in addition to the surviving monomeric α and β chains. They correspond in molecular weight to the species $\alpha\beta$, α_2 , $\alpha_2\beta$ and $\alpha_2\beta_2$, thus confirming the tetrameric character of the enzyme. This distribution of cross-linked components has additional information content, however, for the ab-

sence of the β_2 or $\alpha\beta_2$ species must be taken to indicate that there are probably no β - β contacts in the native tetramer, which restricts the possible symmetrical arrangements to a form of sandwich structure, with an α - α core and one β chain attached to either α (but not to both, because then an $\alpha\beta_2$ species should be generated).

Separation of the α and β chains can be achieved without irreversible damage by dissociation to monomers in 4 M urea, and gel filtration. Removal of the urea from the α chains leads, as shown again by cross-linking, to a mixture of monomers, dimers and multiples of dimers, whereas the β chains form only some dimers.

Biswas and Paulus suggest that this pattern of self-association can follow simply from the sandwich-type structure of the tetramer, if the association sites are in some general sense adhesive, because then the α chains must be divalent, the stronger interaction occurring at the α - α interface, so that the dimeric unit is first formed, and the

β chains univalent, and therefore capable only of forming closed dimers.

The interesting thing now is that not only is there activity in all the α chain species, but all the catalytic and allosteric characteristics are to be found in them. The total regain of activity, however, is no more than about 20%. No enzymatic or regulatory activity is to be discerned in the β chain. When now the renaturation experiment is done in an α - β mixture, the level of reactivation is increased by a factor of three, in a manner that depends on the square of the β chain concentration. The only role that Biswas and Paulus can envisage for the β chain therefore is as an aid to folding of the α subunits—and indeed, the very presence of β chains would of course be expected to create a trap for the native α conformation. It is, of course, conceivable that some other function may yet come to light, and also that the $\alpha\beta$ couple is the product of a single gene, and that a proteolytic scission occurs in the *Bacillus* cell after synthesis.

Immunotherapy of Cancer with Antibody in Rats

THE complexity of anti-tumour immunity, where it exists, is taxing the minds of immunologists who seek to manipulate the immune response to favour the cancer patient. Experiments on animals have shown that T lymphocytes of thymic origin can be effector cells against tumour homografts and that unaided they can apparently kill the tumour. Similarly it has been found that macrophages armed with a product of lymphocytes which have been specifically stimulated can be cytotoxic for malignant cells. More recently it has become clear that a certain class of cell can kill tumour cells if they have been coated with the appropriate anti-tumour antibody but without this sensitizing antibody (LDA) no such lethal effect is evident. The protagonists of each of these killing systems are somewhat doctrinaire and each tends to think that his notions will be of the most use in the immunotherapy of cancer. Hersey, in *Nature New Biology* next Wednesday (July 4), argues the case for LDA and demonstrates that transfer of serum antibody *in vivo* can sometimes contribute to slowing down of tumour growth.

Hersey took a transplantable rat lymphoma which is syngeneic to the PVGc hooded strain of rats and used it to immunize xenogeneic (rabbit), allogeneic (A/gus rats) or syngeneic animals. The xenogeneic and allogeneic antisera were subsequently absorbed *in vivo* in non-tumour bearing PVGc rats to produce specifically anti-tumour antisera. The tumour cells injected into syngeneic recipients were irradiated heavily to prevent their

growth. All sera were titrated for LDA activity using ^{51}Cr -labelled tumour target cells and human peripheral blood mononuclear cells as effector lymphocytes. Only the rabbit anti-tumour serum had any LDA activity after absorption. All the kinds of serum were then injected into PVGc rats before or at various times after implantation of the lymphoma. The survival times were studied and it was found that there was a significant prolongation of life in those animals receiving the absorbed rabbit antiserum. The effect was most marked if the serum was administered prophylactically.

Hersey considers various explanations of his results; for example, that the antiserum in conjunction with complement exerts a direct cytotoxic effect on the tumour cells or, alternatively, that the serum removes antigen-antibody complexes from the plasma of the recipient (by reaction with free combining sites on the antigenic component of the complexes) thus unblocking an effector pathway that was previously inhibited by the complexes. Inevitably he feels that the injected antiserum acts as an LDA *in vivo* as it had *in vitro*.

Hersey goes on to indicate that passive immunotherapy with heterologous antisera might be effective in situations in which LDA activity could be measured *in vitro*. They also seem to show that in a situation in which the host makes no immune response of its own—no anti-tumour antibody was produced in the syngeneic recipients of tumour—an artificial response can be contrived by using an antibody produced in another species of animal.

An interesting story concerning the role of subunit interactions in aldolase has been spun by Chan and his colleagues. They first showed that the activity of the tetrameric enzyme is simply that of an assemblage of identical monomers, by preparing tetrameric hybrids of native and inactive succinylated subunits. They also devised the subterfuge of coupling the enzyme to a column matrix at, on average, a single subunit, and then dissociating the tetramer so as to leave only isolated monomers bound to the column. These proved fully active, though relatively prone to denaturation. It thus seems that there are certainly two (and presumably also intermediate) active forms of aldolase. Chan *et al.* (*ibid.*, 2778) have now demonstrated that in the course of folding these coexist in the reaction mixture.

They have devised assays which will distinguish between the dissociated and tetrameric active forms. Discrimination is again achieved by the use of a critical urea concentration, in this case 2.3 M. In these conditions the tetramer is fully active, but the monomer unfolds. On the other hand, the instantaneous concentration of an active aldolase, monomeric and associated, in a mixture can be assayed by first introducing trypsin, which digests only the unfolded chains (or more precisely, because in optical terms refolding apparently precedes the appearance of enzymatic activity, the inactive population). Thus the kinetics of folding on transfer from concentrated guanidine hydrochloride into water can be followed by these two methods. The upshot is that total enzymatic activity appears according to a more or less first-order process, with no concentration dependence. In urea, by contrast, the kinetics show initially a curved profile, giving place to a lower linear rate, and are strongly concentration-dependent. The data give a convincing fit to a scheme whereby active monomers are first formed, and then associate to the urea-resistant tetrameric state. The identities of the urea-resistant and labile species were verified by determining the elution volumes of the two types of activity from a gel filtration column.

A striking artificial example of thermodynamic trapping of a native conformation by an association process is to be found in the work of Melchers and Messer (*Eur. J. Biochem.*, 35, 380;

1973), who are engaged in sifting the details of the process whereby antibodies to active wild type β -galactosidase of *Escherichia coli* encompass the activation of practically inactive mutant forms. Evidently, the affinity of the antibody for the conformation characteristic of the active state is sufficient to tilt the equilibrium between the active and inactive structures heavily towards the former. The activation occurs on a time scale of minutes, and is associated with an activation energy of about 23 kcal/mole. There is no concentration dependence and evidently the conformational readjustment of the enzyme is the rate-limiting step.

Sorting Out Signals

POLYPEPTIDE chain initiation sites on messenger RNA can be isolated simply by forming test-tube initiation complexes with ribosomes and digesting away the unprotected regions of the RNA with ribonuclease. If the mRNA is appropriately labelled, the nucleotide sequence can be determined by any number of ingenious methods recently developed.

Hitherto such work has been confined to the small RNA viruses, Q β , R17 and f2. Because DNA is not involved in the life cycle of these particular phage RNAs (unlike those of animal RNA viruses in general), the RNA must serve in other capacities besides that of directing protein synthesis: first, of course, the RNA must be replicated; second, control of protein synthesis (both temporal and stoichiometric) can occur only at the level of translation; third, folding of the phage RNA into the mature virus particle may introduce further constraints on the nucleotide sequences of RNA phage messengers in the regions of interest. The nucleotide sequences determining these various functions could conceivably overlap with the ribosome binding sites and be confused with polypeptide chain initiation signals.

Having pointed out the pitfalls in interpreting sequence data using the ribosome binding sites of RNA phage genomes alone, Arrande and Hindley announce in *Nature New Biology* next Wednesday (July 4) that they have now determined the sequence of the corresponding region of the polycistronic message (that which is transcribed by the host polymerase) for the "early" proteins of coliphage T7. The "early" genes are clustered at the left end of the T7 map and a single promoter site for the RNA polymerase has been identified by electron microscopy at the far left of this cluster. Arrande and Hindley have obtained a highly labelled "early" mRNA fragment of about 500 nucleotides by transcription of the phage DNA

EVOLUTION

Crossing the Barrier

from our Plant Ecology Correspondent

As new principles and concepts develop within each of the principal biological disciplines, so there is a tendency to apply these new ideas to the well-worn questions relating to the early development of life on Earth. Palaeontologists, biochemists and cell biologists have already contributed much to knowledge of early living systems; now it seems to be the turn of the ecologist to face some of these long-standing problems.

Stanley, for example, is an ecologist

in vitro (a technique similar in principle to that used in their earlier work on R17 RNA). After digesting the labelled RNA-ribosome complex they were left with a piece of RNA thirty-eight nucleotides long whose sequence they analysed.

The only possible candidate (out of two) for the initiation triplet AUG for the first 9,000 molecular weight protein corresponding to gene 0.3 is that situated at positions 19-21 in the middle of the RNA fragment. This AUG is followed by the pentanucleotide sequence GAGGU which also occurs in the R17 A protein ribosome binding site. Moreover, the tetranucleotide GAGG appears in the corresponding region of the Q β genome. The conservation of the sequence AUGGAGG (U) is consistent with the (logical) prediction that the ribosome recognition site involves more than the initiation triplet AUG and its availability in a hair-pin loop in the messenger RNA. The initiation site has to be distinguished from the triplet for internal methionine residues which is also AUG.

Examination of many more ribosome binding sites, both those within polycistronic messengers, those of both cellular and viral DNA-coded messengers, and those of animal RNA viruses which introduce DNA copies into the cell by means of reverse transcriptase, will be necessary before such vital processes as "modulation" (differential rates of translation of individual cistrons in a polycistronic message achieved by the recognition of specific nucleotide sequences by different initiation factors and/or ribosomes), the piracy of the cellular protein synthesizing apparatus by infectious viruses, and other control mechanisms (for example, the choice between copying, replicating or translating RNA) can be explained (if not yet understood in terms of RNA-protein interactions). The fortunate nucleotide sequencers are going to have a field day sorting out the relevant signals.

Correction

In the editorial "Muscular Dystrophy and the Neurogenic Hypothesis" (*Nature*, 243, 258, 1973) the authors of the letter on page 287 in that issue were given as Kumpel and Dubowitz. The reference should, of course, have been to Gallup and Dubowitz.

who has been looking at the problems of why multicellular organisms arose suddenly at the close of the Precambrian and why the concomitant diversification of living organisms was so explosive (*Proc. Natn. Acad. Sci. U.S.A.*, **70**, 1486; 1973). This rapid radiation of life at the beginning of the Cambrian Period, which began about 570 million years ago, has formed something of a watershed in the geological record and is used as a marker to divide the Precambrian from the Palaeozoic. The rapidity of diversification (taking mere tens of millions of years) is all the more remarkable when it is realized that eukaryotic organisms had already been in existence for more than 1,000 million years, and the advent of sexuality, which might be expected to bring diversification, preceded this explosive radiation by about 250–300 million years.

Stanley suggests that the answer may lie in the trophic structure of these early communities. The relevant ecological principle which Stanley invokes is one formulated by the marine biologist Paine (*Am. Nat.*, **100**, 65; 1966) which states that the addition of a trophic level (that is a predator) to a community tends to promote diversity in the adjacent, lower trophic level (that is among the potential prey). The principle can be seen at work in many ecological systems; the introduction of rabbit grazing to grassland increases plant diversity, whereas the removal of predation in some marine environments has been shown to reduce the diversity of benthic algae.

Stanley conceives of the late Precambrian world as an "all-producer" system, consisting largely of autotrophic, planktonic eukaryotes. This biological monotony could be relieved only by the development of cell-ingesting heterotrophs. This development itself would have been delayed by the slow rate of diversification in a system lacking any true trophic structure. The arrival of such heterotrophs (probably initially bacteria feeders) came in the late Precambrian, and the ability to ingest increasingly large particles would have led to the evolution of algal feeders. The crossing of this "heterotroph barrier", Stanley claims, could have led to the observed burst of diversification. The development of the carnivorous habit required little advancement and, once the food web had arisen, any diversification would lead to further diversification in adjacent trophic levels. Even the development of multicellular organisms could have received an important impetus from the arrival of heterotrophs.

Stanley's theory has much to recommend it because the principles he invokes, if not the system he considers, can be demonstrated experimentally.

One important factor which it neglects is the build-up of oxygen in the Precambrian atmosphere as a result of the dominance of photosynthetic autotrophs. The coincidence of geochemical oxidative processes with the arrival of heterotrophy does suggest some causative interrelationship. Stanley's concept of the diversifying influence of heterotrophy in the Precambrian world may become even more plausible when considered in conjunction with contemporary changes in the early physical environment.

GRAVITATIONAL BIOLOGY

Living in Space

from a Correspondent

GRAVITATIONAL biology occupied only a very small part of the COSPAR programme at Konstanz (May 23 to June 6) and most of the contributions under this heading dealt with ground-based experiments using either centrifuges or klinostats. In the discussions it emerged that the unsatisfactory nomenclature used to describe the conditions of space flight can lead to disagreement as to the relevance of certain attempts at ground-based simulation. The factors neces-

sary for the orientation of cellular processes as well as for the subjective assessment of the vertical need to be clarified. Water flotation, bed rest and forcible restraint in plaster have all been proposed as models for the reduced work load of free fall, and the confusion between "gravity" as a type of force field and "g" as a unit of acceleration leads to extrapolations from centrifuge studies to what has come to be spoken of as the "zero gravity" condition. Perhaps more attention should be paid to the density gradients produced by compression under contact forces rather than to effects attributable to the gravitational field itself.

Dr G. H. Bourne (Yerkes Primate Research Center, Atlanta), attempting to use bed rest as a model for the prolonged weightlessness of space flight, described histopathological changes in monkeys restrained for some months in plaster casts, the stress attributable to the restraint being alleviated by a well-planned social environment. The problems of bone repair during space flights were highlighted by Dr J. R. Beljan (University of California, Davis) who follows, with a collimated proton beam, the course of mineral deposition in a hole drilled through an avian long bone. Repair was much delayed after calcium

Cretaceous Normal Polarity Interval Defined

THE long period of normal geomagnetic polarity in the Cretaceous, discovered by Helsley and Steiner (*Earth planet. Sci. Lett.*, **5**, 325; 1968) and now usually referred to simply as the "Cretaceous normal polarity interval", is likely to prove a useful marker for worldwide correlation. It is all the more important therefore that not only should the interval be delineated as precisely as possible by the data available at any given time but that there should be agreement on its basic definition. Unfortunately, there is now a potential source of confusion in that two rather different definitions have been proposed or implied.

Helsley and Steiner did not offer a formal definition of the Cretaceous normal polarity interval but by referring to "this long period of normal polarity" implied a strict definition—that is, a period of single polarity without internal reversals, however short. From the continental palaeomagnetic results then available they judged the long normal period to stretch from the Upper Albian to the middle Santonian (about 25 million years from about 102 to 77 million years ago). McElhinny and Burek (*Nature*, **232**, 98; 1971), on the other hand, defined a rather longer interval (also from continental data) from 120 to 70 million years ago within which the field was

predominantly normal but which included two known reversed periods at about 80 and 110 million years. Finally, Larson and Pitman (*Bull. geol. Soc. Am.*, **83**, 3645; 1972) used oceanic magnetic anomalies to give a completely normal interval between 111.5 and 84.5 million years ago.

In *Nature Physical Science* next Monday (July 2), Irving and Couillard propose that the Cretaceous Normal (Polarity) Interval as formally defined should refer to that period during which the field is completely normal; in other words, they reject the basis of the definition by McElhinny and Burek. Of course, it is possible that having adopted the strict definition, short reversals within the Cretaceous Normal Interval may be discovered subsequently; but Irving and Couillard argue that such a discovery need not "disturb the chronology". A new analysis of all continental data available up to the end of 1972 then gives 109–82 million years as the best determination of the Cretaceous Normal Interval. Because, however, this agrees so well with the oceanic determination by Larson and Pitman, Irving and Couillard propose limits of 110 and 83 million years as the best compromise currently possible, the uncertainties in these limits being ± 3 million years.

depletion and feeding calcium supplements did not help. Dr L. R. Young (Massachusetts Institute of Technology), in a study of habituation, drew attention to an alternation of dominance between visual and vestibular cues in the perception of motion. Dr T. D. M. Roberts (University of Glasgow) showed with a mechanical model how his new scheme of labyrinth reflexes interact with the neck reflexes to stabilize the trunk, and suggested how the associated sensations might give rise to feelings of disorientation when astronauts move about freely in a large spacecraft.

The COSPAR meeting also provided an opportunity for further informal discussions on plans for the European Spacelab programme for 1980. The Council of Europe Working Party on Aerospace Physiology had recently been told that if it wished to propose a European experiment for any earlier NASA launch, it would have to be one which added no weight to the payload, occupied no space in the vehicle and consumed no power. Undeterred, Dr T. D. M. Roberts put forward a proposal which satisfies all these constraints, cannot be done on the ground, and promises a significant contribution to knowledge.

Information was to be collected on the interaction of neck reflexes with labyrinth reflexes in free fall conditions, using one freely floating astronaut as a subject, while another acts as a force generator, holding on to the spacecraft with one hand while with the other he applies linear accelerations in turn to the subject's head and to his trunk. Because the subject's arms are not engaged either in locomotion or in their own support against gravity, reflex movements should appear, and these should be visible from the ground over the existing television link. When this proposal was submitted in detail to NASA, yet a fourth insuperable constraint was revealed—the experiment must not occupy any additional astronaut time! A fully instrumented version of the experiment is to be included in the ESRO Spacelab programme.

NON-DESTRUCTIVE TESTING

Warsaw Meeting

from a Correspondent

At the Seventh International Conference on Non-Destructive Testing, held at Warsaw from June 4 to 8, Drs P. D. Hanstead and R. C. Wyatt (Central Electricity Generating Board, Bristol) described how ultrasonic images of discontinuities in manufactured components can be visualized directly, without scanning, using relatively simple techniques. Pulsed ultrasound of a few MHz frequency, passing through a sample immersed in a liquid, is brought

to a focus in a transparent medium, which is illuminated stroboscopically. Visualization is, in one instance, by the Schlieren method and, in the other, by photoelasticity. In the second case, where the transparent medium must be a solid, images formed by shear waves as well as by longitudinal waves can be observed.

Drs S. A. Lund and P. Jensen (Danish Welding Institute) dealt with the scanning of welds by ultrasonic shear waves, which enter the test sample obliquely, using what they call the "P scan", a modification of C scanning, in which a flaw indication is recorded in a position corresponding to that of the defect, yet independent of that of the transducer. They claim that the method provides an indication of weld defects with a definition better than that given by radio-

graphy. Dr H. Jünke (Ingenieurhochschule, Berlin-Wartenberg, DDR) gave an account of a method of testing safety glass using ultrasonics. A glass sheet immersed in water is scanned by Lamb waves, which are induced by mode conversion, and their group velocity measured. There is a decrease of about 6% in this velocity in "toughened" glass as compared with untreated glass.

In radiology, Drs K. F. Sinclair, H. A. Zagorites and K. A. Zimmerman (Xetex Inc., Belmont, California) described a method of scanning using a technique which they term "scintillography", in which no film is used. The signal from the received radiation is observed as a trace on a chart, which moves in synchronism with the examined object. The method can be used with X ray, γ ray, and neutron beam sources. Applica-

QSOs and Intergalactic Gas

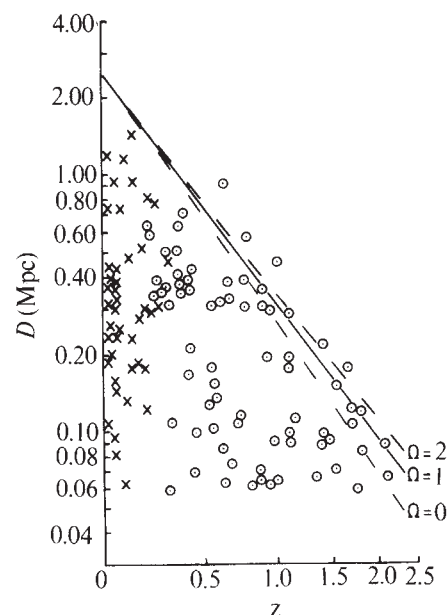
IN *Nature Physical Science* next Monday (July 2) Strom compares observations of the radio polarization of quasars with measurements of their angular size, and suggests from the trends in the data that quasars are expanding into a significant density of intergalactic gas.

It is generally agreed that to obtain regular behaviour, quasars must be separated into those with flat radio spectrum and those with steep spectra. Flat spectrum quasars, believed to be recently exploded objects, show a wide scatter in strength, size and radio polarization, presumably because of random circumstances in their formation. The mature steep spectrum quasars, on the other hand, have angular sizes which decrease with increasing redshift z , as is expected, and furthermore show depolarization due to internal Faraday effects which increases at high z .

Different astronomers have put forward different explanations for this trend in the polarization data. According to Strom it follows naturally from the supposition that the quasars are expanding into an intergalactic gas. The density of the gas increases at great distances, as $(1+z)^3$, and decelerates the explosion, forcing the radio source to be contained within a volume which decreases with z . On average, therefore, the gaseous products of the explosion will have higher density, and will cause greater Faraday effects, if the quasar is at high z .

This hypothesis also succeeds in accounting for a curious feature of the angular size data. In a Euclidean universe, the angular size of an object decreases with redshift as z^{-1} . The geometry of a universe with relativistic cosmology is less simple, and for the most probable models the angular size stops decreasing and may actually increase

again, at redshifts of about 0.25, well within the range covered by quasars. Miley's compilation (*Mon. Not. R. astr. Soc.*, **152**, 477; 1971) shows no signs of this "elbow" in the universe, and suggests that the steep spectrum quasars seem to follow the Euclidean law with no further cosmological factors. Such arguments assume all quasars to have the same intrinsic linear size. Strom's hypothesis, that their linear size varies according to the surrounding medium, though it cannot yet be put on a quantitative basis, does succeed qualitatively in removing a curious paradox.



Component separation, D , calculated on the basis of an Einstein-de Sitter ($\Omega=1$) metric and plotted against the redshift, z (the axes are $\log D$ and $\log(1+z)$), for $D > 60$ kpc. X, Radio galaxy; O, quasar. Expected upper envelope for radio components decelerated by an intergalactic medium, $D \propto (1+z)^{-3}$.

tions include the testing of welds and the detection of foreign bodies in frozen food. In another radiological contribution, Dr G. C. Wheeler (General Electric Co., Schenectady) described a variable energy accelerator with an output of up to $25,000 \text{ r min}^{-1}$ at 10 MeV, suitable for testing steel up to 20 inch thick.

A theoretical contribution on eddy current methods was given by Drs V. G. Gherasimov, V. V. Soukhorukov, and N. P. Rtishcheva (Power Engineering Institute, Moscow). They described an analogue process for simulating defects in ferromagnetic materials in order to predict crack depths very reliably. On the more immediately practical side, Drs W. A. Decker and D. L. Waidelich (University of Missouri) described the design of an electric field probe emitting short high energy pulses into dielectrics; thus defects and metallic inclusions can be detected in plastic specimens up to 12 mm thick. A special feature is a correlation technique which, by making use of two receiving probes in different positions, keeps noise levels low.

A novel method of non-destructive testing was described by Dr H. Licht (Rheinisch-Westfälische Technische Hochschule, Aachen), in which Lamb waves are propagated in steel plates as a result of alternating Lorentz forces set up by eddy currents induced in the presence of a steady magnetic field. The transducer, in the form of a coil, does not have to be in contact with the material tested.

COSPAR

Plenty in Prospect

from a Correspondent

ONE of the highlights of the COSPAR meeting at Konstanz (May 23-June 6) was the series of contributions on the results from ERTS-1, the Earth Resources and Technology satellite. This satellite is in a 570-mile-high polar orbit and takes television pictures of 115-mile square regions of the Earth's surface in four colours—green, red and two in the near infrared. These pictures are proving of immense use for regional surveys of soil and terrain type, vegetation, geology, land use and water sedimentation and turbidity. The problems associated with mapping the more inaccessible regions of the world—for example, the Amazon Basin—are considerably lessened because the near orthographic view from ERTS-1 minimizes the distortion problems of conventional aerial mapping. Dr A. A. Jackson (University of Botswana, Lesotho and Swaziland) gave a dramatic account of how ERTS imagery is enabling Lesotho to make the best of its limited and scattered rainfall. In that country, which has few roads and limited communication, areas where

rain had fallen and grass grown could easily be picked out from the images, thus enabling Lesotho to optimize its grazing potential and minimize the problems of overgrazing and subsequent soil erosion. Regions suitable for re-afforestation and diamond prospecting could also be identified.

Dr C. Sagan (Laboratory for Planetary Studies, Cornell University) revealed some of the latest information from the Mariner 9 mission to Mars. Dark streaks on the planetary surface were found to change with wind variations; these streaks were caused by the scattering of light by the larger dust particles which are blown about more easily. Interestingly the data obtained from ground based observations of the dark markings on the planet give a perfect picture of the Martian wind and weather patterns during the past century. The sizes of the two satellites Phobos and Deimos have now been measured accurately; given in terms of the radii of spheres with the same area these are $5.7 \pm 0.5 \text{ km}$ and $10.9 \pm 1.5 \text{ km}$ respectively.

Problems of lunar chronology are still very much in evidence. Dr H. W. Hinnert (NASA, Washington) questioned why the Moon, formed 4.6 aeons ($4.6 \times 10^9 \text{ yr}$) ago, should suffer many large impacts 3.9 aeons ago and then after considerable volcanism become relatively inactive 3.1 aeons ago. He also pointed out that the distinctive isotropic and chemical dissimilarities with Earth indicate that the Moon did not fission from Earth, the Earth-Moon system thus being the result of a dual planet formation or a capture mechanism.

Returning to the Earth's atmosphere, Dr G. Witt (University of Stockholm) discussed the problems of deciding what forms the nucleation centres for noctilucent cloud crystals; it is still not clear whether they are dust particles (blown up from below or incident from space) or large water molecules such as hydronium H_3O^+ (H_2O)₂. Dr M. Dubin

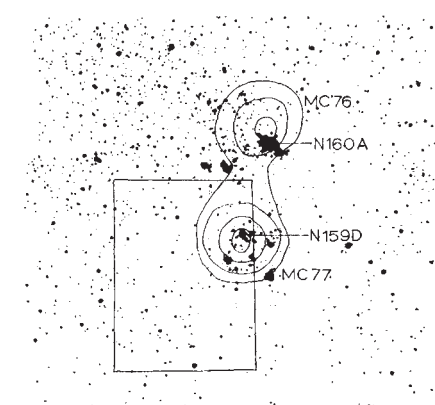
(NASA, Washington) put forward the thesis that the blue haze seen sporadically on Mars is analogous to terrestrial noctilucent clouds and is formed by water condensing on accreted cosmic dust which is being blown out of the Solar System. The fact that the Sun is surrounded by a dust cloud is well established, but the density variation of this cloud is still a matter of considerable discussion. Preliminary results presented by Dr R. K. Soberman (Drexel University, Philadelphia) and Dr Matha S. Hanner (Dudley Observatory, Albany) from their meteoroid and zodiacal light detectors on Pioneer 10 indicate that this problem is nearing a solution.

Thinking of the future, Professor L. Biermann (Max Planck Institute, Munich) discussed the possibilities of sending space probes to intersect comets, the two candidates being comet Grigg-Skjellerup in 1976 and comet Encke in 1980. Slowing down the spacecraft to extend the period during which it is in close proximity to the comet is particularly important. Experiments to investigate the size and structure of the nucleus, to measure, using mass spectrometers, the chemical composition of the coma and to study the solar wind-comet interactions were all propounded. Dr S. I. Rasool (NASA, Goddard) led the conference on a trip round the Solar System by describing NASA's plans for future missions which include the July 1975 Viking spacecraft which will soft land on Mars and will, by analysing soil samples using a gas chromatograph-mass spectrometer, look for life on that planet. Another project discussed was the 1973-74 gravity-assisted mission to Venus and Mercury which will endeavour to take about 7,000 television pictures of Mercury to a resolution of 1 km and 5,000 during the Venus flyby. Equipment will also be carried to measure the surface temperatures, topographies and atmospheric compositions as well as the planetary masses, spins and radii.

Candidate for LMC X-1

THE X-ray source in the Large Magellanic Cloud (2U0540-69) may be identified with the radio sources MC76 and MC77, according to Byrne and Butler (see *Nature Physical Science* next Monday, July 2).

Excluding 30 Dor, these two radio sources are the brightest in the LMC at 6 cm and 11 cm. Like five other known LMC sources, they form a double object with one thermal and one non-thermal component. They are identified with H II regions N 160 A (MC76) and N 159 D (MC77) and MC76 may be variable. At 55 kpc, the separation of the two sources is 120 pc.



Error box of LMC X-1 and 6 cm radio contours of MC76 and MC77 superimposed on B plate of the region.

Entropy and Demography

F. N. ARUMI

School of Architecture and Planning, and Physics Department, University of Texas, Austin, Texas

A socio-physical theory describing the land distribution patterns of a system in demographic equilibrium is developed in this article.

THE behaviour of social systems, regardless of species, must conform to some natural laws in the same way as non-social systems. This article explores the feasibility of using equilibrium statistical theories in the description of large population aggregates. It describes the density distribution of land in a theory that can be formalized in terms of a disorder (entropy) function. The resultant distribution is a rigorous consequence of the postulates. As in statistical mechanics, the distribution is derived using general arguments that are quite independent of the mechanisms responsible for bringing about the equilibrium state.

In this approach it is the inhabited land that is distributed among the population. In order therefore to make discrete counting of land possible, I postulate the existence of a quantum of personal area. The rules for counting which I use assume the distinguishability of the population members and the indistinguishability of quanta. The total number of quanta is always greater than or equal to the population and I therefore postulate that all the inhabited land assigned to a given population is occupied.

A demographic system is defined here as an isolated, steady population residing in a given and fixed land area. A system in demographic isolation cannot exchange population or land with other demographic systems. The state of a demographic system is defined by specifying the total population and the total inhabited land. A configuration of the state is any possible distribution of the additional quanta among the population members. A microconfiguration in turn is any distinguishable arrangement of individual members of the population that yields a given configuration. A microstate is the generic term for all microconfigurations of a state regardless of the corresponding configuration. The total number of microstates is the sum of all the microconfigurations of all the possible configurations of a state. The disorder or entropy of a configuration or of a state is the natural logarithm of the number of microconfigurations or microstates respectively.

Number of Microstates

If the population and area of a state are given by N_0 and A_0 respectively, the number of additional quanta of area is given by

$$m = A_0/a - N_0 \quad (1)$$

where a is the magnitude of the quantum of area. The number of microstates, that is, the total number of ways in which m quanta can be distributed distinguishably among the N_0 individuals, is given by the combinatorial expression

$$\Omega = (m + N_0 - 1)! / m!(N_0 - 1)! \quad (2)$$

The disorder, or entropy, of the state is by definition

$$S = \ln \Omega \quad (3)$$

Expected Density Distribution

The total number of ways in which a population member may occupy quanta is given by the number of distinguishable

ways there are of placing $(m+1-k)$ quanta into (N_0-1) individuals. The answer is given by the same combinatorial expression

$$g_k = (m + N_0 - 1 - k)! / (m+1-k)!(N_0-2)! \quad (1 \leq k \leq m+1) \quad (4)$$

Therefore, the fractional population in the k th level is

$$n_k / N_0 = g_k / \Omega \quad (1 \leq k \leq m+1)$$

where $\langle n_k \rangle$ is the expectation value of the population in the k th level. Using equation (2), this expectation value can be expressed as

$$\langle n_k \rangle = (m + N_0 - 1 - k)! m! N_0 (N_0 - 1) / (m+1-k)!(m + N_0 - 1)! \quad (1 \leq k \leq m+1) \quad (5)$$

This expression satisfies the two basic conservation rules:

(i) conservation of individuals

$$N_0 = \sum_{k=1}^{m+1} \langle n_k \rangle \quad (6)$$

(ii) conservation of area

$$A_0/a = m + N_0 = \sum_{k=1}^{m+1} k \langle n_k \rangle \quad (7)$$

Table 1 illustrates the counting procedure leading to equations (2) and (4). Figure 1 illustrates the behaviour of equation (5).

Large Argument Approximations

For large arguments ($N_0, m \gg 1$), equation (5), which is exact, can be approximated by a functional expression after using the relation

$$(n+x)! \simeq n! n^x \quad (n \gg 1)$$

Thus

$$\langle n_k \rangle \simeq N_0^2 / m(m + N_0)^k \quad (8)$$

The behaviour of this approximate expression is compared with the exact results in Fig. 1. This result also satisfies the conservation rules provided that the upper limit of the sums is considered to be effectively infinite.

Theorem of Greatest Disorder

The expression for the entropy (equation (3)) can also be simplified. Applying Stirling's formula and then in succession equations (8), (6), (7) and (8), the expression for the entropy is recast into the form

$$S = N_0 \ln N_0 - \sum_{k=1}^{\infty} \langle n_k \rangle \ln \langle n_k \rangle \quad (9)$$

In this form, the distribution function (8) is readily recognized to correspond to the configuration that renders the entropy of the state an extremum.

Following the standard method of Lagrange multipliers to solve for the distribution that renders S an extremum, the demographic "temperature" of the system turns out to be

$$T = a / \ln \{ (m + N_0) / m \} \quad (10)$$

and for the partition function of the microcanonical ensemble

$$f = 1 / \{ \exp(\beta a) - 1 \} \quad (11)$$

where $\beta = 1/T$. That a temperature function can be defined is a natural consequence of the use of equilibrium statistical arguments.

The behaviour of the average area per person ("internal" area) is displayed as a function of demographic temperature in Fig. 2. Real demographic systems, of course, are not in demographic equilibrium. This theory, however, is approx-

Table 1 Counting Procedures Leading to Equations (2) and (4)

State		kn_k^r =No. of cells to person					r or k	n_k^r =population in level k and configuration r					Ω^r	Ω	$g_k=\sum_r n_k^r \Omega^r N_0$					
N_0	m	1	2	3	4	5		1	2	3	4	5			1	2	3	4	5	
3	1	2	1	1			1	2	1				3	3	2	1				
	3	4	1	1			1	2					3							
		3	2	1			2	1	1	1			6	10	4	3	2	1		
		2	2	2			3		3				1							
5	0	1	1	1	1	1	1	5					1	1	5					
	4	5	1	1	1	1	1	4					5							
		4	2	1	1	1	2	3	1			1	20							
		3	2	2	1	1	3	2	2	1			30	70	35	20	10	4	1	
		3	3	1	1	1	4	3		2			10							
		2	2	2	2	1	5	1	4				5							

kn_k^r represents the number of cells a given person may have; in this case each person is assigned a number label, for example, for $N_0 = 3$, $m = 3$ and configuration $r = 2$, person 1 has three cells, person 2 has two cells and person 3 has one cell.

$\Omega^r = \text{No. of ways configuration } r \text{ occurs}$. $\Omega = \text{No. of microstates} = \sum_r \Omega^r$. $g_k = \text{No. of ways a person may occupy } k \text{ cells}$. Conservation of area: $m + N_0 = \sum_k kn_k^r$. Conservation of people: $N_0 = \sum_k n_k^r$.

imately applicable to systems in quasiequilibrium where the relaxation time is short compared with the typical time over which the ratio N_0/A_0 changes.

Empirical Test

In order to test the theoretical distribution I shall recast equation (8) into a form more readily matched with accessible empirical data. Cumulative population and area at level r are the respective sums of the population and area in all levels from $k = 1$ to $k = r$.

$$N_r = \sum_{k=1}^r n_k = N_0 \{1 - (m/(m + N_0))^r\}$$

$$A_r = a \sum_{k=1}^r k \langle n_k \rangle = A_0 \{1 - [1 + rN_0/(m + N_0)][m/(m + N_0)]^r\}$$

where N_r and A_r are the cumulative population and area respectively. An individual in the k th level has a personal area equal to ka . The population density in this level is therefore $p = 1/ka$. Using equation (1) the cumulative area and population can be expressed as functions of density, depending on the total population (N_0) and the total area (A_0)

$$N(p) = N_0 \{1 - \exp(\beta/p)\} \quad (12)$$

$$A(p) = A_0 \{1 - (1 + N_0/A_0 p) \exp(-\beta/p)\} \quad (13)$$

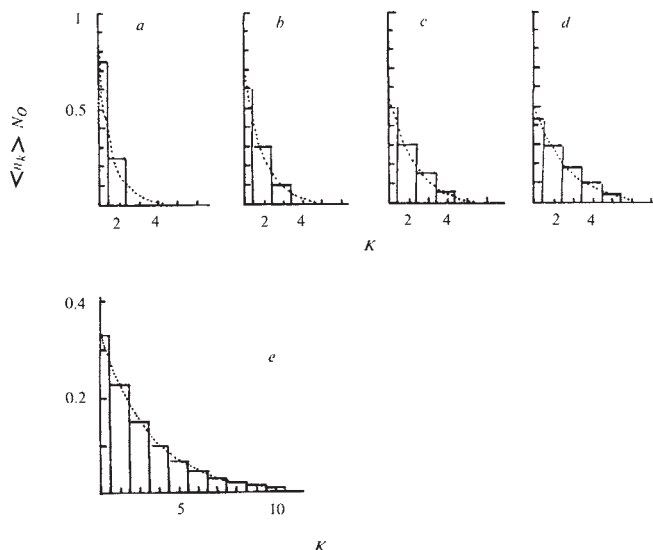


Fig. 1 Expectation value, $\langle n_k \rangle$, as a function of density level, k . —, Behaviour of the exact value, equation (5). . . ., Analytical approximation (equation (8)). a, $m=1$; b, $m=2$; c, $m=3$; d, $m=4$ (all for $N_0=4$); e, $m=100$ ($N_0=50$).

where $\beta = 1/T$, and the “temperature” T is given by equation (10).

I chose the urban population of the United States as the demographic system to be tested. As of 1960, the latest complete Census Data available at the start of this investigation, there were 5,409 towns listed with a population greater than 1,000 people¹. This population is referred to here as the urban population. Smaller towns are not included because it was not possible to assign them a well defined area of occupation from the available information.

The demographic system is therefore comprised of the sum total of the population and area of these towns. The state parameters of the empirical system are

$$N_0 = 113,762,784 \text{ people} \\ A_0 = 33,887 \text{ mile}^2$$

and the density distribution ranges from 24 people mile⁻² to 40,138 people mile⁻². The population, area and density of each town are then arranged in descending order of density. The empirical cumulative population and area are calculated by summing the individual values of population and area successively as the density scale is descended. The resulting values, plotted as a function of density, are shown in Fig. 3.

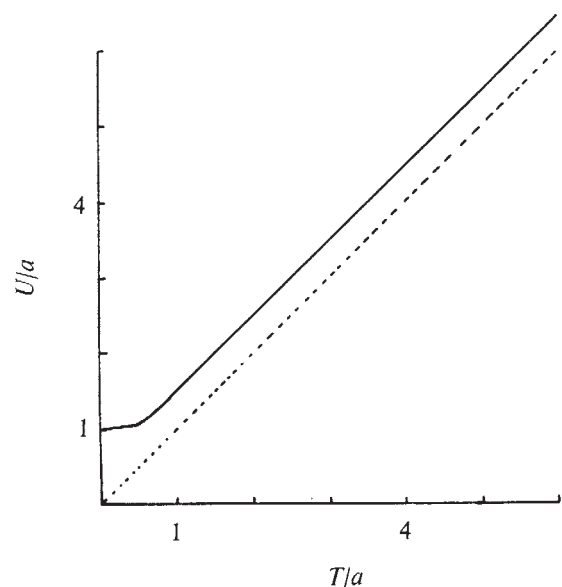


Fig. 2 The average (“internal”) area per person ($A_0/N_0 \equiv u$) as a function of the demographic “temperature”. For uncrowded systems, $A_0/N_0 \gg a$, the “temperature” is equal to the average area per person. As the “temperature” drops below a , the standing room only starts developing. See equations (13) and (1). $\exp(-a/T) = 1 - aN_0/A_0$; $u/a = (1 - \exp(-a/T))^{-1}$. For $T/a \gg 1$, $u/a \approx T/a + 0.5$; for $a/T \gg 1$, $u/a \approx 1$.

The theoretical distribution was generated by equations (12) and (13) using the corresponding state parameters.

The value of the quantum of area was used as a fitting parameter. It was found that no appreciable change in the theoretical predictions could be generated for values of $a < 620 \text{ foot}^2$ ($\sim 62 \text{ m}^2$). The distribution obtained with values of $a < 620 \text{ foot}^2$ yields the closest fit with empirical data. The validity of this upper limit is further reinforced by the fact that the highest density point in the data is less than $1/620 \text{ foot}^2$.

The theoretical distributions are shown as solid curves in Fig. 3. The agreement between empirical and theoretical density distribution of area is excellent. The agreement for the distribution of people is quite good although not as close as the one for area.

The disagreement may arise from a combination of facts. The demographic system studied is not truly isolated, as the 5,409 towns considered are in contact with the countryside and the data correspond to a period of rapid flux of rural population to urban sectors. The lower levels of population in the higher density regimes may be a consequence of the sprawling of American towns. The rate of sprawling may be higher than the relaxation rate of demographic systems seeking equilibrium. The system described is not in equilibrium. As such, a departure from the theoretical equilibrium is to be expected. Conceptually the theory remains ambiguous in the definition of inhabited land (A_0). A more precise definition will provide the theory with additional fine tuning capabilities.

Cross-cultural Validity

The formalism of the theory is independent of cultural, economic or political conditions of the demographic system. This implies that the same rules of land distribution should be detectable in any demographic system in the world. The predicted distribution therefore should be tested against empirical data from a variety of demographic systems that exhibit different cultural, economic and political conditions.

Demographic Contact and Clustering

A corollary of the theorem of greatest disorder states that the optimum entropy of two demographic systems brought into contact is always greater than or equal to the sum of the individual optimum entropies of the two separate systems. This means that clustering into single groups is more likely than staying as distinct and separate subgroups. This clustering tendency has been reported by behavioural scientists in their controlled observation of animals². According to this theory therefore urban-suburban systems and twin cities should exhibit higher entropies than the sum of the component subsystems. This prediction can be tested by obtaining small grained data of well established cities with suburbs.

Limits of Growth

A system with standing room only value corresponds to a state where the number of quanta is equal to the population ($m=0$ in equation (1)). For a fixed land area, however, the entropy of the system is a maximum for a population equal to half of the "standing room only" value. If this is interpreted as being the occupationally most favourable size, then the maximum population would be equal to half of the "standing room only" value ($N_{\text{max}} = A_0/2a$). Calhoun reports³ that under controlled conditions, a mice population, whose standing room only value was to be 4,000, stopped growing at about 2,000. The agreement of this result with the prediction of this theory is remarkable. The relationship between the two uses of the expression "standing room only" value, however, remains to be determined. Verification of this phenomenon in human populations would provide an important test of the theory. Should it not be verified, however, it would not reflect negatively on the theory. Rather the interpretation of the population with the greatest entropy as the maximum population would have to be discarded.

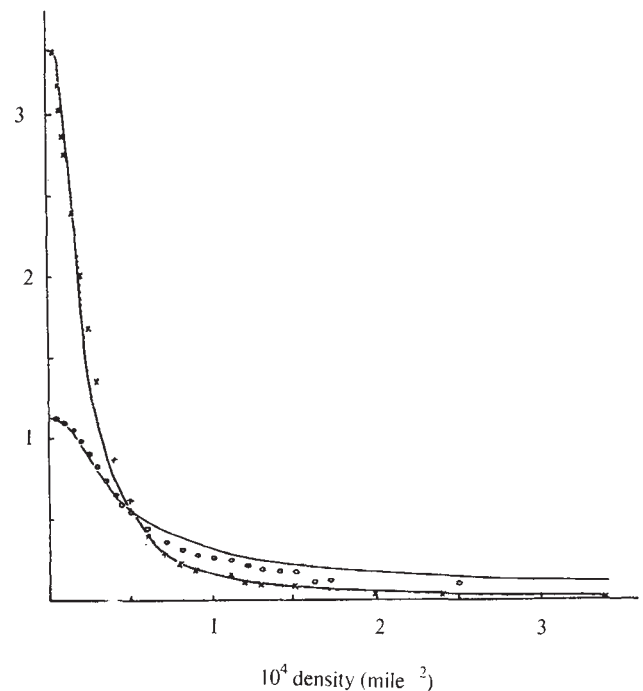


Fig. 3 Empirical area A_0 (10^4 mile^2) ($\times$) and population N_0 (10^8 people) ($\circ$) as a function of density from the urban population data from the 1960 US census. Solid curves represent the theoretical predictions as generated by equations (13) and (12) respectively.

To carry out this test a demographic system with the following characteristics would have to be identified. (1) It would have to be demographically isolated; (2) there would have to be apparent stability of the population; and (3) the supply of food and shelter would have to be such that for the lack of these necessities would not be responsible levelling of the population.

Redistribution

This article has shown the potential usefulness of applying the methods of equilibrium statistical theories to the study of social systems. The theory was formalized in terms of a disorder function and the fundamental theorem states that a given population will redistribute itself within the inhabited land so as to maximize this function.

The predicted distribution was tested very successfully against empirical data taken from the urban population of the United States. The quantum of area was found to have an upper limit of about 650 foot^2 . The possible relationship of this concept with that of personal space remains to be determined. More tests should be carried out to provide additional verification or to shed light on fundamental fallacies.

The internal density distribution of population in a single town should be compared with the theoretical prediction. This test may provide the means to identify more precisely the nature of the constraints of what constitutes the inhabited land area of a system.

I thank Mr Mark Schott for compiling and processing the empirical data and Drs M. E. Dakes, P. Antoniewicz, T. Griffy, D. Poston, and De Long for their comments on the initial manuscript. This work was financed in part with a University of Texas Special Research grant.

¹ *Census of Population, 1960, Characteristics of the Population Part A, 1* (US Bureau of the Census).

² McBride, G., *Theories of Animal Spacing Behavior and Environment, The Use of Space by Animals and Man* (edit. by Esser, A. H.) (Plenum, 1971).

³ Calhoun, J. B., *Space and the Strategy of Life, Behavior and Environment, The Use of Space by Animals and Man* (edit. by Esser, A. H.) (Plenum, 1971).

Reduction in the Concentration of Nerve Growth Factor in Mice after Sialectomy and Castration

I. A. HENDRY & L. L. IVERSEN

MRC Neurochemical Pharmacology Unit, Department of Pharmacology, University of Cambridge, Cambridge

Surgical removal of the submaxillary glands in adult mice causes a profound drop in the plasma and tissue concentrations of nerve growth factor, and a reduction in the activity of tyrosine hydroxylase within sympathetic ganglia. NGF may be the protein which fulfils the retrograde flow of information from the axon terminal to the cell body.

THE recent development in this laboratory of a sensitive radioimmunoassay for the active subunit of mouse salivary gland nerve growth factor (NGF) has enabled detailed studies to be performed on the very low concentrations of this protein in tissues and plasma. Plasma concentrations of NGF in adult mice parallel the marked sex difference in NGF concentration in the submaxillary glands, and it was suggested that the normal tissue and plasma content of NGF is derived by secretion from the salivary glands^{1,2}.

In the studies described here, we found that surgical removal of the submaxillary glands in adult mice caused a profound drop in the plasma and tissue concentrations of NGF. There was, furthermore, a reduction in the activity of the enzyme tyrosine hydroxylase within sympathetic ganglia, which correlated with the reduction of the NGF content of the peripheral tissues innervated by these ganglia. On the basis of these findings a hypothesis is proposed concerning the trophic role of NGF in the sympathetic system.

Mice of the Swiss albino strain aged approximately 50 d were given Oxo diet 41B and water *ad libitum*. Submaxillary glands were removed under ether anaesthesia, the main vessels tied with silk ligatures and the wounds closed using Michel's clips. Sham operations consisted of freeing each lobe of the submaxillary gland and then closing the wound. The NGF was donated by Dr D. C. Edwards of the Wellcome Research Laboratories, and was a pure preparation of the β -subunit as described by Varon *et al.*³. For some experiments NGF was bound to aminocellulose powder as previously described¹.

Nerve growth factor was assayed by the previously described radioimmunoassay method¹. Tyrosine hydroxylase was assayed by a radiochemical procedure involving the estimation of ³H-L-dopa formed from side chain labelled ³H-L-tyrosine⁴.

For NGF assay, tissue samples were homogenized in 10 volumes of 0.05 M veronal buffer, pH 8.6, containing 0.9% sodium chloride, 0.5% bovine serum albumin, and 0.1% sodium azide. Blood samples for NGF assay were taken from the inferior vena cava of anaesthetized mice into a heparinized syringe, and diluted 1:3 (v/v) with the veronal buffer described

above. Sympathetic ganglia were removed using a dissecting microscope; they were stored frozen (-20°C) in 10 μl of distilled water before the assay of tyrosine hydroxylase activity⁴.

Submaxillary Gland and Plasma NGF

There was a marked reduction in the concentration of NGF in the plasma after the removal of the submaxillary glands in both male and female mice (Fig. 1). In male animals the fall in plasma concentrations of NGF had a half time of 9 d, and the concentration fell to a minimum of 15% of the normal adult control level by 33 d. After this there was a fairly rapid recovery to approximately normal plasma concentrations, which were reached by 60 d post-operatively.

In female animals the effects of sialectomy were qualitatively similar, with a half time for the fall in plasma concentrations of 6.5 d. The minimum value was reached earlier, however, and was not relatively as low as the male (approximately 25% of adult control values).

As normal male animals have a higher concentration of NGF in plasma than females, the minimum absolute values for plasma NGF after sialectomy were similar in males and females. The recovery phase in female mice was more prolonged than in males, plasma concentrations of NGF returning to normal only after 120 d.

This return to normal occurred in the absence of any significant regeneration of the NGF stores in the submaxillary or other salivary glands (Table 1). The concentration of NGF in the remaining salivary gland tissues (including parotid and sublingual) was slightly higher than that found in unoperated

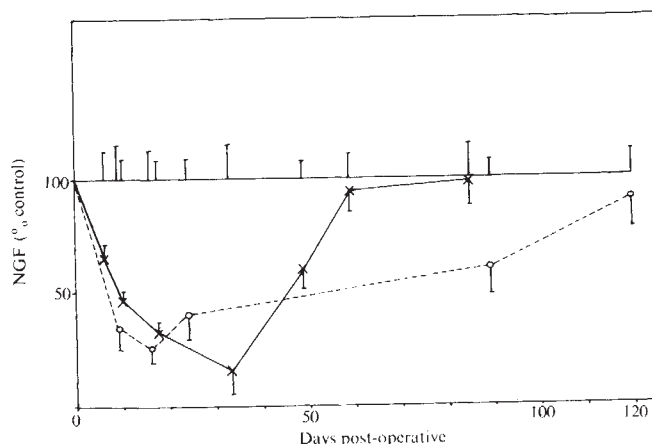


Fig. 1 Effects of removal of both submaxillary glands on the plasma concentrations of NGF in adult mice. Results expressed as means $\pm$ s.e.m. for groups of four–six mice. Control (100%) values were 2.6 ± 0.63 ng β -NGF ml^{-1} for female animals (O) and 11.5 ± 0.38 ng ml^{-1} for males (x).

Table 1 NGF Content of Mouse Salivary Glands in Normal and Sialectomized Adult Mice

Tissue	NGF (ng mg ⁻¹)	
	Female	Male
Control submaxillary gland	88 ± 10.1	209 ± 30.4
Control "residual salivary gland"	0.14 ± 0.02	0.43 ± 0.014
"Residual salivary gland" 17 d post-op.	1.15 ± 0.16	0.50 ± 0.03

The submaxillary gland was removed rapidly after death in the control animals, and 17 d before death in the sialectomized animals. The remaining salivary gland tissue (including parotid and sublingual glands) was assayed as a whole and classed as the "residual salivary gland".

animals. This may be attributable to some very slight regeneration of the submaxillary gland, or to the synthesis of NGF in granuloma tissue as reported by Levi-Montalcini and Angeletti².

Submaxillary Gland and NGF in Other Tissues

There was a gradual reduction of the NGF content of heart and superior cervical sympathetic ganglia in female mice after the removal of the submaxillary gland; but the fall in these tissues was slower than that observed in the plasma (Fig. 2). The NGF content of superior cervical ganglia fell to 50% of the control values after 15 d and the heart content reached 50% of the control concentration after 40 d. The lowest concentrations of NGF in these tissues were reached at a time when the serum concentration of NGF was already returning towards normal.

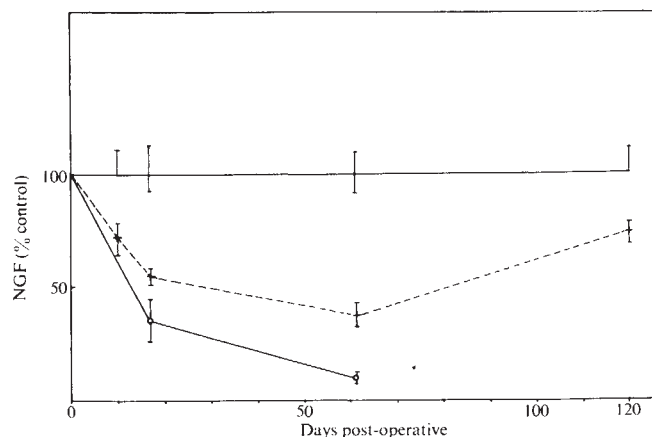


Fig. 2 Effects of removal of both submaxillary glands on NGF concentrations in the heart (+) and superior cervical ganglion (○) content of adult female mice. Values are means ± s.e.m. for groups of six animals and are expressed as percentages of the control values (0.26 ± 0.09 ng mg⁻¹ for heart and 0.05 ± 0.012 ng per ganglion).

Response of Sympathetic Ganglia

Tyrosine hydroxylase (T-OH) is an enzyme specifically located within adrenergic neurones in the sympathetic ganglion⁵ and its activity in such ganglia is increased after administration of NGF to infant mice⁴. The activity of this enzyme in sympathetic ganglia from sialectomized animals was examined. A detailed study of the changes in T-OH activity in the superior cervical ganglia after the removal of the submaxillary glands was undertaken in female mice. A reduction in the activity of this enzyme was found within 10 d of the operation, and ganglionic enzyme activity reached a minimum of about 30% of normal adult values at 32 d, and slowly recovered to near normal levels by 120 d (Fig. 3).

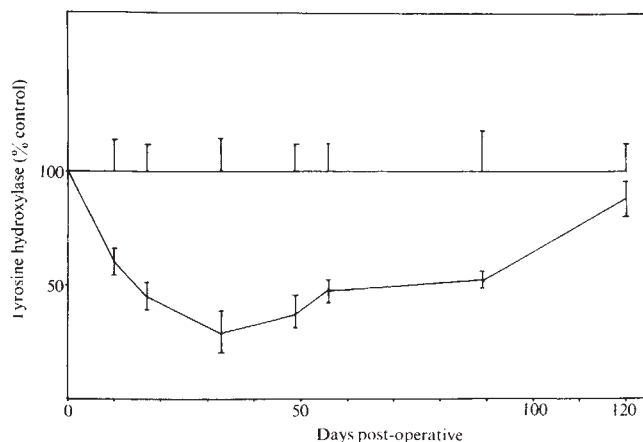


Fig. 3 Effect of the removal of both submaxillary glands on the total tyrosine hydroxylase content of superior cervical ganglia of adult female mice. Values represent means ± s.e.m. for groups of four to six mice. Mean control value (100%) was 15.8 ± 2.48 pmol dopa h⁻¹ per ganglion.

In male animals there was also a fall in the T-OH activity of superior cervical ganglia after the removal of the submaxillary glands, but in this case the enzyme activity remained significantly depressed even at 56 d after sialectomy, when the plasma concentrations of NGF had returned to normal (Table 2). Thus, it seemed that the ganglionic T-OH activity might not depend directly on the plasma concentration of NGF, but corresponded better with the concentrations of NGF found in the tissues. The reduction in T-OH activity in superior cervical ganglia after the removal of the submaxillary glands may be caused by several factors. To determine whether the circulating concentrations of NGF, or contact of the adrenergic neurones in the ganglia with their end organs, which include salivary glands, would be the more important factor, the submaxillary gland was removed in some animals on one side only, and the contralateral side served as control. In these circumstances there was no reduction in plasma NGF; in fact there was a slight rise, probably as a result of damage inflicted on the intact gland during surgery (Table 2). The superior cervical ganglia on the operated side of such animals thus suffered from the removal of a major end organ but there was no change in the concentration of circulating NGF to which the ganglia were exposed. There was a fall in T-OH activity in the ganglia on the operated side, while the values in ganglia from the contralateral control side were unaffected (Table 2). Thus, the T-OH activity of the ganglia seems to depend more on contact with their end organ than on the circulating concentrations of NGF.

Table 2 Effects of Removal of the Submaxillary Gland and on the Concentration Plasma NGF and Superior Cervical Ganglion Tyrosine Hydroxylase Activity in Adult Male Mice

	Plasma NGF (ng ml ⁻¹)	Superior cervical ganglia tyrosine hydroxylase (p mol ⁻¹ per ganglion)
Sham operated	6.89 ± 0.05	18.7 ± 2.32
Left submaxillary gland removed		
17 d post-op.	11.80 ± 2.50 *	
Right side control		21.1 ± 2.18
Left side operated		10.6 ± 1.32 †
Both submaxillary glands removed		
17 d post-op.	2.43 ± 0.55 †	8.9 ± 0.99 †
56 d post-op.	6.98 ± 1.02	9.2 ± 1.01 †

Values represents means and standard errors for groups of six male mice. Plasma NGF concentration and tyrosine hydroxylase activity were assayed as described in the text.

* $P < 0.01$.

† $P < 0.001$ when compared with sham operated values.

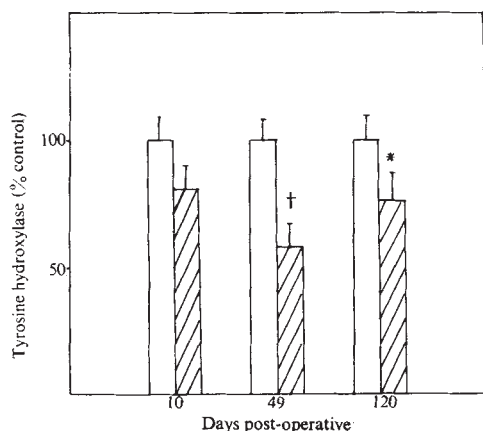


Fig. 4 Effects of removal of both submaxillary glands on the total tyrosine hydroxylase content of the stellate ganglion of adult female mice. Values are expressed as % of control tyrosine hydroxylase activity and standard errors (vertical bars) for groups of four to six animals. □, Control; ▨, operated. * $P < 0.05$; † $P < 0.005$ when compared with corresponding control values. Mean control value (100%) was 18 ± 1.85 pmol dopa h^{-1} per ganglion.

Some of the adrenergic neurones in the superior cervical ganglia innervate the submaxillary glands, so the reduction in T-OH activity might be a result of retrograde degenerative effects in such neurones after axotomy. To test this, histological studies⁶ of the ganglia were undertaken to determine if the fall in enzyme activity was associated with any damage to the ganglia or a loss of cells resulting from chromatolysis after axotomy. There was no significant change in the numbers of cells in the ganglia after sialectomy (Table 3), although there was a small but non-significant decrease in the average cell diameter.

Table 3 Cell Numbers in Superior Cervical Ganglia after Removal of the Submaxillary Gland

	Cell No.	Cell diameter (μm)
Control	$12,913 \pm 588$	14.9 ± 0.77
Submaxillary gland removed	$13,368 \pm 1,591$	14.0 ± 0.27

Effect of the removal of the submaxillary gland on the cell numbers and cell diameters of the superior cervical ganglion. The results express the means $\pm$ s.e.m. for groups of six animals. Ganglia were fixed in 2.5% glutaraldehyde stained with cresyl violet and cell numbers and cell diameters estimated as previously described⁶.

The effect of the removal of the submaxillary gland on the superior cervical sympathetic ganglion might be a result of the removal of the end organ *per se*, or of the removal of a trophic influence on the ganglion neurones, normally exerted in a retrograde fashion by the large amounts of NGF in the innervated submaxillary gland. The large reduction in T-OH activity found in the superior cervical ganglion after the removal of the submaxillary gland may reflect an intense effect of this type exerted by the very high concentrations of NGF in the mouse salivary gland. The relationship between T-OH activity and the NGF content of the end organ was accordingly examined in a different group of sympathetic neurones, those in the stellate ganglion. The postganglionic axons of the neurones in this ganglion should not be damaged by the removal of the salivary glands, and as they innervate the heart, this tissue was used to monitor changes in the end organ content of NGF as a representative sample of the tissues innervated by this ganglion. There was a fall in the T-OH activity of the stellate ganglion after sialectomy, and the extent of this fall correlated temporally with the fall in NGF concentration in the heart (Fig. 4). This finding lends further support to the view that end organ NGF may act as a retrograde trophic influence on the neurones in sympathetic ganglia.

Removal of Salivary Gland in Neonatal Mice

It was impossible to obtain an adequate survival rate in neonatal mice subjected to bilateral sialectomy. Animals more than 6 d old, however, survived unilateral submaxillary gland removal and grew normally. Animals operated at age 6 d and killed at 12 d showed a fall in the T-OH activity of the superior cervical ganglion on the operated side (Fig. 5). The decrease in enzyme activity was not as great as that observed in adult animals after unilateral sialectomy, and this may reflect the lower concentration of NGF in the neonatal submaxillary glands.

The role of NGF in the developing sympathetic nervous system is still unclear. Salivary glands of neonatal mice contain only very small amounts of NGF; it is possible that the major¹ source of NGF to the foetus derives from the maternal circulation. NGF labelled with ¹²⁵iodine is able to cross the placenta and is accumulated in the foetal tissues (unpublished observations).

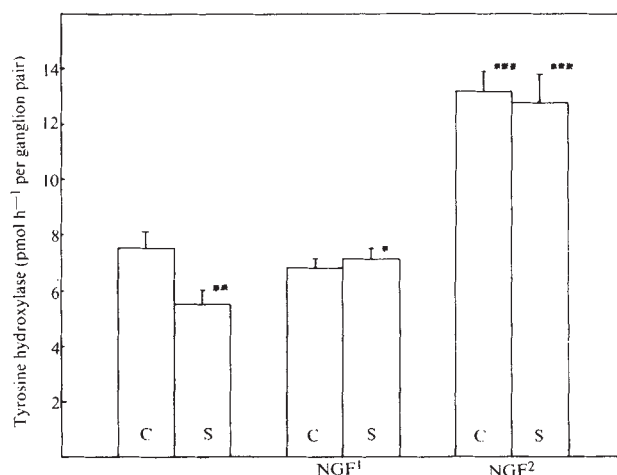


Fig. 5 Results of administration of NGF to neonatal mice after unilateral removal of submaxillary gland on tyrosine hydroxylase in superior cervical ganglia. Animals were operated at age 6 d and treated with daily injections of NGF as described in the text for 6 d post-operatively. Values represent means $\pm$ s.e.m. for groups of six animals. Mean control value (100%) was 7.4 ± 0.22 pmol dopa h^{-1} per ganglion. C, Value from control ganglion on non-operated side; S, value from ganglion on the side of removal of submaxillary gland; NGF¹, daily injections of solid phase NGF; NGF², daily injection mixture of solid phase and soluble NGF as described in text. * Does not differ from control but differs from sialectomized animals, $P < 0.05$; ** differs from controls, $P < 0.05$; *** differs from controls, $P < 0.001$.

An attempt to examine the effects of lowering the maternal NGF concentration on the development of the sympathetic nervous system in the foetus was undertaken by removing the salivary glands in adult female mice. Unfortunately this operation produced a temporary infertility in these mice; in twenty-five animals none became pregnant within 100–120 d of the operation, by which time the maternal and foetal serum concentrations of NGF had returned to values not significantly different from normal.

In order to assess the physiological function of the NGF in animals in which the salivary gland had been removed, an attempt was made to replace NGF in the site of removal of the salivary gland. Unilateral submaxillary gland removal was performed in 6-d-old animals and they were subsequently treated daily with an injection of a suspension in saline of 2 μg β -NGF (bound by diazotization to 20 μg of cellulose) per gramme body weight into the site of removal of the sub-

maxillary gland. The NGF-cellulose preparation released approximately 0.3% of the bound NGF d^{-1} and acted as a depot source of NGF. This injection of NGF prevented the reduction in ganglionic T-OH activity after the unilateral removal of the submaxillary gland. In order to assess the role of the localized action of the slow release of NGF compared with the effect of soluble NGF a similar experiment was performed using a mixture of soluble NGF and cellulose-bound NGF. A mixture of soluble NGF and cellulose-bound NGF was made so that 30% was bound to cellulose and 70% was soluble. The same total daily dose of NGF was given to the animals as in the previous experiment. In this case there was a marked stimulation of the tyrosine hydroxylase activity in both control and operated sides, but the difference in activity between these was again eliminated (Fig. 5). Thus, it would seem that the depot injections of NGF stimulated only the ganglion innervating the injected side, whereas soluble NGF had a more general stimulating action on sympathetic ganglia.

Castration, NGF and Tyrosine Hydroxylase

Thirty days after castration, NGF concentrations were markedly reduced in submaxillary glands, plasma and heart (Table 4). The fall in tissue NGF contents was again accompanied by a significant reduction in the T-OH activity of both cervical and stellate ganglia (Table 4).

Table 4 Effects of Castration on NGF and Tyrosine Hydroxylase in Mouse Tissues

Concentration of NGF	No.	Control	Castrated
Submaxillary glands	4	100 $\pm$ 29.9	7.8 $\pm$ 0.4 †
Plasma	6	100 $\pm$ 12.0	40.0 $\pm$ 8.0 ‡
Heart	6	100 $\pm$ 19.0	5.0 $\pm$ 1.0 ‡
Tyrosine hydroxylase activity			
Superior cervical ganglion	6	100 $\pm$ 7.0	67.0 $\pm$ 7.1 ‡
Stellate ganglion	6	100 $\pm$ 11.0	69.0 $\pm$ 7.2 *

Animals were killed 30 d after castration, and the tissues assayed for NGF concentration and tyrosine hydroxylase activity as described in text. Value are means $\pm$ s.e.m. for the number of animals indicated, and are expressed as per cent of control values from similar numbers of unoperated animals (for control values see Figs. 1-4 and Tables 1 and 2).

* $P < 0.05$.

† $P < 0.01$.

‡ $P < 0.005$, when compared with corresponding control value.

Implications

Removal of the submaxillary glands from adult mice produced a reduction in the serum concentration of NGF in both sexes, indicating that the normal serum concentrations of NGF are dependent on the presence of intact submaxillary glands, which contain the principal stores of NGF in normal adult animals. After removal of the submaxillary glands, however, other as yet unidentified sources of NGF gradually emerged and were capable of maintaining normal serum concentrations. NGF increases in concentration in some fast growing mesodermal tissues, for example granulomas² and in the sarcomas 37 and 180 (ref. 7). The sources of NGF other than the submaxillary glands may reflect a generalized synthesis of NGF by mesodermal tissues rather than synthesis by any one particular alternative tissue. There was no marked increase in the NGF content of parotid or sublingual glands, nor in a variety of other glandular tissues after sialectomy. The alternative source tissues responsible for NGF production after sialectomy must, however, be responsive to male sex hormones, as the normal difference in the circulating concentrations of NGF in male and female animals was restored 2 months after sialec-

tomy. The importance of circulating sex hormones in controlling NGF production offers another experimental means of altering tissue and plasma concentrations in adult animals. Castration also leads to profound reduction in the NGF concentration of mouse salivary glands, plasma and other tissues. This reduction in NGF production was again accompanied by a fall in T-OH activity in the superior cervical and stellate ganglia, although in this case the neurones in these ganglia did not suffer any surgical damage.

The regulation of the activity of the enzymes tyrosine hydroxylase and dopamine- β -hydroxylase in the superior cervical sympathetic ganglion and in the adrenal medulla of adult animals is partially attributable to trans-synaptic influences⁸⁻¹². We have presented evidence here that the regulation of the activity of at least one of these biosynthetic enzymes, T-OH, may also depend upon the NGF content of the innervated end organ. There is a marked reduction in the activity of this enzyme in the superior cervical ganglion after the removal of the submaxillary gland, which may reflect the removal of a large pool of NGF from the field of innervation of that ganglion. The results of the experiments in which the T-OH activity of the stellate ganglion was compared with the NGF content of the heart after sialectomy seem to support this hypothesis.

The possibility of a retrograde flow of information from end organs to their innervating neurones has been under investigation for many years. In 1934 Hamburger¹³ demonstrated an increased degeneration of motor cells in the developing chick central nervous system after limb amputation. Prestige¹⁴⁻¹⁶ found a decrease in the number of cells in the dorsal root ganglion and in the ventral horn after amputation of the hind limb in *Xenopus*. In the brain, Cragg¹⁷ has shown that lesions placed in specific areas may cause both orthograde and retrograde trans-synaptic effects.

A mechanism for the transmission of such information from the periphery has been postulated by La Vail and La Vail¹⁸ who found that horseradish peroxidase injected into the optic tectum of chicks was transported in a retrograde manner to the cell bodies of the ganglion cells in the retina at a rate of about 72 mm d^{-1} . They suggested that this retrograde movement of protein from axon terminal to the cell body might represent a mechanism by which neurones responded to their target areas. The results presented here are consistent with such a hypothesis. In the sympathetic nervous system NGF may be the protein which fulfils this retrograde-information-transfer role.

The ability of NGF to reverse the effects of end organ removal on the T-OH activity of cells in sympathetic ganglia is consistent with the hypothesis that these effects are attributable to the removal of the trophic action of NGF. The different effects observed between the cellulose bound NGF and the soluble NGF are similar to effects observed by Levi-Montalcini and Hamburger^{19,20}, with different types of sarcoma growing in chick embryos. The small cell type of mouse sarcoma produced only low levels of NGF, and here only the sympathetic ganglia innervating the tumour were found to be enlarged. On the other hand, the large cell type of tumour had high NGF activity and these tumours caused a massive increase in all the sympathetic ganglia in the embryo.

The present hypothesis might also explain why adrenergic neurones in infant animals are more responsive to exogenously administered NGF than in adults. It would be expected that adrenergic neurones would be most sensitive to administered NGF during early stages of development, before their axons had established contact with NGF sources in their end organs. One might also postulate that during normal development only those adrenergic neurones which succeeded in establishing contact with their end organs would receive the supply of NGF needed for their continued survival. This could explain why the administration of NGF to young animals leads to increases in both the size and number of cells in sympathetic ganglia. The apparent hyperplasia evoked by NGF might represent in fact the artificially sustained survival of large numbers of

adrenergic neurones that would normally have died through failure to make contact with their end organs.

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Salts in the Sea

EGON T. DEGENS*

Department of Chemistry, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Salty hydrothermal solutions are discharged along rift valleys. Their composition gives insight into the chemical evolution of the sea.

THE first 10⁹ yr of Earth history have left no stratigraphic record from which to decipher the chemistry of the primordial ocean. As a result, one can only speculate on its composition using criteria such as equilibrium models¹⁻³, geochemical balance calculations⁴⁻⁹, or evolution of mineral facies in the Precambrian^{10,11}.

Here I use a new indicator for the old problem. My discussion is based on chemical analyses of hydrothermal fluids from the East African rift region, primarily Lake Kivu. I believe that these waters have received their salts under geological conditions similar to those that exist when waters are released to the sea along oceanic rifts and that this process was essentially the same during early stages in the development of the Earth's crust. Therefore, their chemistry will give us clues to the kind and quantity of salts that have been discharged from the oceanic lithosphere to the sea during geological times.

Geology and Hydrography

Lake Kivu is situated about 100 miles north of Lake Tanganyika, at the highest point of the East African rift valley 1,500 m above sea level. The greatest water depth measures 500 m; reducing conditions prevail below about 50 m.

The lake is surrounded by active volcanoes, and is fed by salty hydrothermal springs; stable isotope data indicate that the hydrothermal reservoir is charged by local rain water. This agrees with sedimentological studies of a 12 m

core section of the past 25,000 yr; periods of hydrothermal activities and heightened volcanism seem to coincide with pluvial times¹²⁻¹⁴. These rain waters, enriched with volcanic CO₂, accumulate their salt load by percolating through the rift rocks at elevated temperatures, and are finally discharged into the deep lake as thermal springs.

An examination of the lake's structure reveals a unique stratification pattern. Temperature and salinity increase in a stepwise manner with depth (Fig. 1). Such steplike profiles, observable both in nature and the laboratory, are a result of convection¹⁵. The individual water layers or cells are well mixed within themselves and separated by sharp boundaries. The development of such water cells permits retention of dense hydrothermal fluids in the deepest part of the lake. As long as a certain flux of heat and salt is maintained, the layers will stay intact with little or no mixing with surface waters. Calculations on the residence time of the salts, using sodium as a reference standard, yield an age of 350 yr.

Below the thermo and halocline (>50 to 60 m), the analyses indicate linear relationships among most elements. The pH of the water is 9.2 at the surface and <6 at depths

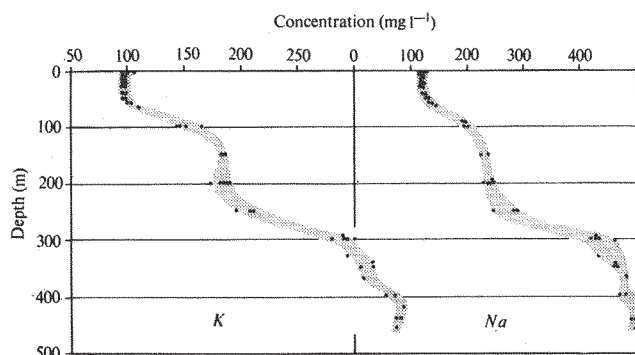


Fig. 1 Distribution of sodium and potassium in Lake Kivu waters¹²⁻¹⁴. Samples came from stations as much as 60 km apart. The narrow spread in concentration at a given depth indicates that Kivu waters are structurally defined.

* Present address: Geologisch-Paläontologisches Institut der Universität Hamburg, 2000 Hamburg 13, Von-Melle-Park 11.

Table 1 Water Analyses of Lake Kivu

Depth (m)	Ca	Mg	K	Na mg l ⁻¹	Cl	SO ₄	ΣCO ₂	P	Si	N(NH ₄) μg atmos l ⁻¹	N(NO ₃)	N(urea)
0	5	87	97	122	55	24	572	1	231	18	2	0.4
100	64	147	145	192	86	25	1,280	19	424	487	0.4	2
200	83	182	179	245	110	166	1,850	33	428	1,314	0.3	2
350	111	394	315	465	205	214	5,850	53	1,056	5,460	0.5	5
440	113	417	338	487	215	220	7,040	55	1,226	7,105	0.3	3

Table 2 Water Analyses of Kabuno Bay

Depth (m)	Ca	Mg	K	Na mg l ⁻¹	Zn	F	ΣCO ₂	P	Si	N(NH ₄) μg atmos l ⁻¹	N(NO ₃)
5	35	97	117	125	<0.5	1.4	n.d.*	0.5	65	3	0.2
20	67	355	281	283	1.4	int.†	n.d.	1	589	30	0.1
40	127	478	336	363	2.1	2.6	n.d.	11	1,636	461	0.2
120	92	803	416	471	4.5	2.3	6,420†	66	2,122	552	0.1
140	91	823	427	481	7.2	1.9	7,130	70	2,240	565	0.1

* Not determined. † Measured at 130 m. ‡ Iron hydroxide interference; 55 mg Fe l⁻¹.

greater than 250 m. A representative profile from the centre of the lake is presented in Table 1. Kivu water below 300 m will release ~1.5 l of CO₂ and 0.4 l methane l⁻¹ water at NTP. Because concentrations of CO₂ and methane are below saturation at depth, these gases are also trapped by the strong stratification pattern of the lake. Other gases detected include nitrogen, hydrogen sulphide, a few light hydrocarbons, and possibly hydrogen.

Sodium and chlorine levels in the deep water are definitely controlled by hydrothermal emission; however, the extent of hydrothermal control for other elements was not discernible at first. For example, magnesium and calcium may precipitate as carbonates; heavy metals can be removed as sulphides close to the point of hydrothermal discharge; and effects of microbial activity on CO₂, SO₄, CH₄, PO₄, NH₃ and trace metal concentrations are only poorly understood. Within a few hundred years many reactions might indeed proceed. Data from an expedition to Lake Kivu in March 1972 added more conclusive information.

In 1948 a lava flow reached the northern shore of Lake Kivu and dammed off a portion of the lake 15 km long, 8 km wide, and reaching 150 m in depth. This part, known as Kabuno Bay, is presently connected to the main Kivu basin by a narrow, shallow channel. Since this incident, hydrothermal waters accumulated and almost completely filled the bay. The chemical analyses of some ions and nutrients in the bay water are shown in Table 2. CO₂ and sodium values are the same as those in deep Kivu basin water. The other elements show only moderate differences between the two sets of analyses.

I think that the chemical composition of deep Lake Kivu and Kabuno Bay water roughly mirrors, in kind and proportion, the original salt composition of hydrothermal solutions emanating from active rift zones. Based on this assumption, the type and quantity of salts released to the ocean via hydrothermal channels can be calculated.

Hydrothermal Salt Flux

To estimate the oceanic hydrothermal salt flux, a major ion must be found which is derived principally from hydrothermal processes rather than from weathering of crustal material, and is not appreciably lost from the oceanic system by processes such as precipitation or evaporation. Chlorine fits the description best. On the basis of geochemical balance calculations¹⁶, only 0.5% of chlorine in the

oceans actually comes from the weathering of igneous rocks.

In Table 3 (column A) the concentrations of salts in Lake Kivu have been adjusted for a chlorinity of 1.935%. The ΣCO₂ is included in this Table to emphasize the abundance of free CO₂ in the hydrothermal system; this amount certainly varies from hot spot to hot spot. Converting the major cations present in the sea to oxides would yield a value of ~5 kg cm⁻² of the Earth's total surface which would be equivalent to a sediment layer about 20 m thick over the whole Earth¹⁷. A corresponding calculation for an ocean filled with hydrothermal water (Table 3, column A) will yield a shell around the Earth of ~260 m.

Calculations for some trace constituents enriched in Kivu waters are also presented in Table 3. The value for iron has been corrected for sulphide formation close to the point of hydrothermal discharge. Converting the trace metal ions to oxides, these oxides could cover the Earth's surface in a layer 20 m thick.

Table 3 Determination of Hydrothermal Salt Flux

	Ocean *	Kivu	A	B
		g kg ⁻¹		
Na	10.76	0.48	44.23	66.35
K	0.39	0.43	39.62	59.43
Ca	0.41	0.09	8.29	12.44
Mg	1.29	0.82	75.56	113.34
Cl	19.35	0.21	19.35	29.03
SO ₄	2.71	0.22	20.27	30.41
HCO ₃	0.72	7.13 †	657 †	986 †
		μg kg ⁻¹		g kg ⁻¹
Si	4	63	5.80	8.7
P	0.07	2.1	0.19	0.29
N	0.5	7.9	0.73	1.10
F	1.4	2.3	0.21	0.32
Fe	<0.1	40	3.69	5.54
Mn	<0.01	1	0.09	0.14
Zn	<0.01	7.2	0.66	0.99
Cu	<0.005	0.2	0.02	0.03

* To obtain the total mass (kg) of each element in the ocean multiply the numbers in this column by 1.37×10^{18} .

† Expressed as ΣCO₂.

A = Conc (Kivu) × $\frac{(\text{Cl ocean})}{(\text{Cl Kivu})}$

B = 1.5 A.

Sinks for hydrothermal chlorine include recent and ancient sediments as well as crystalline rocks derived from metamorphism and granitization of former sediments. Chlorine fixed in sediments in the form of interstitial waters, salt deposits and ordinary minerals constitutes about 25% of the amount present in the sea^{5,8,16}; excess of chlorine in granites, diorites, syenites and metamorphic rocks above the amount released from weathered basalts yields another 25%. The sum total of hydrothermal chlorine is thus about 50% above the present seawater chlorinity. Column B in Table 2 has been adjusted for these contributions; recalculating the thickness of the oxide shell around the globe, a figure of slightly more than 400 m is obtained of which heavy metals comprise 30 m. Alternatively, if all hydrothermal metals were extracted in the form of oxides at a constant rate for the Earth's entire history, this rate would be $\sim 10^{-5}$ cm yr⁻¹.

Chemical Evolution of Seawater

Assuming that chlorine was added to the sea as a salt rather than as a volatile constituent, and that the remaining ions in the hydrothermal solutions generated along rift zones have an approximate Kivu-type distribution, then initially seawater was magnesium and carbonate rich. Over geological time seawater evolved to sodium chloride dominance; but how did this occur?

In following the concept of Sillén¹⁷, I propose that reactions between the solutes in the sea and the minerals in sediments are responsible for the steady state conditions. Work on deep sea sediment cores¹⁸ indeed indicates a marked decrease of magnesium and potassium from interstitial solutions and a corresponding increase in calcium; however, interstitial chloride and sodium concentrations remain constant. Potassium may be incorporated into illites and glauconites, and magnesium may enter into clay minerals such as chlorite and montmorillonite. Magnesium may also be removed as sulphate and carbonate.

Interactions between seawater and minerals frequently lead to liberation of hydrogen and calcium ions, in exchange for potassium, iron and magnesium ions¹⁻⁴. The increased availability of calcium in turn promotes a more effective extraction of magnesium in the form of dolomite and high magnesium calcite, and eventually results in massive limestone production. In this manner the initially large reservoir of free CO₂ can be gradually depleted until the carbonate equilibrium which presently exists in the ocean is established. A fraction of the carbon dioxide is fixed as reduced carbon by inorganic or biological means. Carbon isotope data indicate that—at least since the Cambrian—about one-third of the incoming hydrothermal CO₂ is removed in the form of reduced (=organic) carbon and two-thirds in the form of carbonates¹⁹.

Sulphate can be deposited as magnesium and calcium sulphate. With the advance of anaerobic bacteria capable of reducing sulphate, sulphides provide an even more effective sink.

Geological data support this line of reasoning: clay minerals in Precambrian and early Palaeozoic shales are chiefly chlorites and illites, while kaolinite and expandable clays are more abundant in younger sediments²⁰; Ca-Mg carbonates show compositional changes with age starting with high magnesium members in the Precambrian and developing towards almost pure CaCO₃ in the Cretaceous^{21,22}; carbonaceous chondrites—considered to have originated in a primitive hydrosphere—contain the low temperature minerals magnesium-iron chlorites, epsomite (MgSO₄·7H₂O), limonite, ferroan magnesite (breunnerite) and gypsum²³; and there is a lack of rock salt deposits in strata of Precambrian age.

Kivu sediments contain many of the mineral phases of early rocks¹²⁻¹⁴. If they are subjected to diagenesis, banded magnesium-iron silicates, iron oxides, siderite, dolomite,

sulphides and chert deposits will develop, closely resembling Precambrian iron formations.

Flux of Interstitial Water

Recent sediments contain on average 90% water by weight (96% by volume). The amount of water in ancient sediments varies between 10 and 50% by weight; this variability is determined by texture, structure, composition, age and compaction history of the rock. Seawater is thus constantly lost to the sediment column²⁴. By calculating the mean annual sedimentation rate, the average thickness of sediment material (compacted to zero % H₂O) which accumulates in 100 m.y. in the ocean basins is around 650 m; expressed in terms of a sediment layer around the entire Earth's surface, a value of 460 m is obtained. Assuming that these rates have been fairly constant throughout the Earth's history, a sediment mantle, about 20 km thick, would cover the Earth. This sediment mass would be about twice the mass of the present continental crust.

In the process of mountain building, sediments become folded, metamorphosed, and granitized. Sedimentary water is expelled and is transformed into hydrothermal fluids. These fluids carry certain ions and gases away from the rock system, and eventually return, after many transformations, to their starting place, the ocean.

In order to calculate the quantity of water involved in orogenic events, I assume that at the time of complete or partial melting a sediment still holds about 50% water by volume. Dividing the interstitial water volume by the volume of water contained in the world ocean—with a mean ocean depth of 3,729 m (ref. 25)—about 8 times the volume of the present oceans has passed through the continental crust and oceanic trenches, that is, one complete cycle takes 500 m.y. In contrast, rivers renew the ocean every 50,000 yr.

Assuming that marine sediments were the primary source of sedimentary and crystalline rocks composing the continental crust, it follows that weathering and erosion simply recycle ancient marine deposits. The most significant factor controlling the concentration of marine salts is seawater-mineral interaction which determines the rate at which individual ions are removed from the hydrosphere either syngenetically, diagenetically, or orogenetically. Because of the long residence time of meteoric water in geosynclines and the continental crust, and the elevated temperatures to which geosynclinal sediments are commonly subjected, it seems to me that seawater is presently close to equilibrium with continental rocks. The position of equilibrium will shift gradually in response to the input of new salts from rift activities. As the mass of continents increased with time, the significance of hydrothermal rift salt contributions on seawater composition grew less and less.

Overall Model

Two types of hydrothermal water can be recognized. The first is associated with active rift zones, and the second is generated from geosynclinal sediments in the process of mountain building. Both are principally of meteoric origin. Ascending rift water supplies new salts to the ocean, whereas rising geosynclinal water chiefly recycles salts derived from ancient sediments. A Soxhlet-extraction analogy adequately describes the principles involved. A rising pulse of hydrothermal water reacts with oceanic and continental rocks picking up salts and gases. Some of this dissolved material is removed from the fluids in response to pressure, temperature or chemical changes. The remainder of the solution is released to the hydrosphere and atmosphere, thereby bringing the cycle to a close. Thus, the composition of the Earth's atmosphere and hydrosphere is interrelated with rift and geosynclinal activities.

The enrichment of sodium and potassium in the conti-

mental crust relative to the oceanic crust is attributable to the addition of alkalis to the ocean in the vicinity of rifts, the removal of potassium by clays, and the involvement of sodium-rich formation water during granitization. The sources of hydrothermal ore deposits are heavy metals and sulphur previously concentrated by sedimentary processes. Further, hydrothermal wall rock alteration may explain some of the mineral and rock assemblages observed in rift zones (for example, greenstones and greenschists).

Seawater began as a carbonate solution rich in magnesium. It gradually evolved to a sodium chloride dominance by a series of mineral equilibria. The excess in CO_2 was removed by carbonate deposition and inorganic and biological CO_2 reduction. Steady state conditions were probably established at the end of the Precambrian, at which time organisms began to form a carbonate shell.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Non-Primary Magmas and Dubious Mantle Plume beneath Iceland

BASALT lavas dredged from the mid-Atlantic ridge south of Iceland exhibit a conspicuous correlation of minor element chemistry with distance from Iceland¹. This also happens to be a correlation with altitude, those lavas which contain the higher concentration of incompatible elements (and the higher Fe/Mg and Na/Ca ratios) being collected from greater heights above the general level of the ocean floor. The lavas are predominantly quartz-normative and most contain phenocrysts of olivine, augite and plagioclase.

Schilling¹ has rejected differing degrees of fractional crystallization as an explanation of these relationships. He prefers a process of mixing of two primary magmas, the one generated by partial melting of depleted upper mantle spreading from the ridge, and the other generated by partial melting of undepleted deeper mantle rising as a plume beneath Iceland.

Here I summarize the evidence that these lavas are not primary magmas, but may have suffered extensive low and high pressure fractional crystallization. I re-examine the proposition that a varying degree of fractional crystallization is responsible for the variation in lava chemistry and offer an alternative interpretation of the observations, without recourse to mantle plumes, in terms of a hypothesis of magma genesis at the upper mantle-transition zone boundary.

Experimental petrology of quartz-normative basalts² has shown that they are not in equilibrium with olivine at pressures greater than 2 to 5 kbar. They cannot, therefore, be primary magmas derived from a peridotite upper mantle at greater pressures because olivine (and enstatite) will be resi-

dual crystalline phases in that material when the partial melting occurred. Olivine and calcic plagioclase are, moreover, not stable together at pressures greater than 8 to 10 kbar (ref. 3) so the phenocryst assemblage cannot be explained as residual from the source mantle. This point has been made previously with respect to ocean floor tholeiite⁴ when I pointed out that olivine would not be present at the liquidus of the more siliceous of such rocks beyond ~2 kbar or the more basic beyond ~7 kbar. This has been confirmed recently by experiment⁵ and enstatite does not appear at the liquidus of these rocks at any pressure (the pigeonite which appears transiently in one sample investigated by Kushiro and Thompson has an $\text{Mg}/(\text{Fe} + \text{Mg}) = 0.845$, much more iron-rich than presently suggested for residual upper mantle mineral compositions).

The values of $M (=100 \text{ Mg}/(\text{Mg} + \text{Fe}))$ in the lavas change in such a way on passing northwards along the ridge that they would be in equilibrium with an olivine with $M=83$ to 78 (if all iron is present as FeO) or 86 to 81.5 (if 20% of all iron is present as Fe_2O_3) assuming the Roeder-Emslie⁶ empirical relationship to hold. These indicated olivines are approximately twice as ferriferous as the olivines of postulated fertile upper mantle ($M=88$ to 92) in the case of iron-rich basalts allegedly derived from the undepleted mantle plume, and also iron-rich relative to the olivines of postulated⁷ depleted upper mantle ($M=96$ to 94). In simple terms, these basalts are too rich in iron relative to magnesium to represent primary magmas from likely upper mantle compositions and an interval of olivine fractionation is indicated in order to decrease their M values relative to the true primary magmas⁴.

A true primary magma derived by substantial partial melting at pressures of 10 to 25 kbar as suggested by Schilling (ref. 1, Fig. 4) should be at a temperature above its liquidus on arrival at low pressure and, moreover, its composition

should bear no special relationship to that of a liquid in simultaneous cotectic equilibrium with several crystalline phases at low pressures. Yet it is clear that most of the basalts under discussion were erupted with phenocrysts of olivine, augite and plagioclase present. They seem to have had the temperature and composition of liquids in cotectic equilibrium with these three crystal species at low pressures and this has been confirmed experimentally in the case of the 1783 Lakagigar fissure eruption⁸ which seems to belong to the same petrographic group. Such relationships are a mandatory consequence if low pressure fractional crystallization had modified the magma compositions.

Nevertheless, basaltic liquids can be in equilibrium with olivine, augite and plagioclase over a considerable temperature range. It would be expected that those lavas erupted at the higher levels, which have higher Na/Ca and Fe/Mg ratios and higher Ti, K, P and REE than those erupted at lower levels, would have the lower liquidus temperatures and must, therefore, have been erupted at lower temperatures in order to contain the observed phenocrysts. This can easily be verified by experiment. Such a relationship accords with a model in which greater amounts of low pressure fractional crystallization affect those magmas which have to rise to higher levels before eruption. The temperature relationship is the reverse of that predicted by the plume model (ref. 1, Fig. 4).

The way in which the erupted magmas might be related to the true primary magmas has been explored from the theoretical point of view^{2,4} and substantiated by studies of an actual province⁹. Once olivine, augite and plagioclase are fractionating together at low pressure, or when eclogite fractionates at high pressure, large reductions in the residual liquid volume are possible without dramatic changes in the character of the residual magma, except with respect to its concentration of incompatible elements. The basalts under discussion seem to have been erupted from the condition of olivine-augite-plagioclase fractionation, but the basalts richest in the rare earth elements do not show the relative depletion in the lighter rare earth elements which is expected when significant fractionation of clinopyroxene has occurred.

The $(La/Yb)_{EF}$ ratio in the basalts poor in incompatible elements and collected south of 61° N (ref. 1, Table 1) is 0.28, indicating very substantial enrichment of heavy REE in these magmas relative to chondritic ratios. In the basalts rich in incompatible elements and collected north of 63° 50' N, the $(La/Yb)_{EF}$ ratio is 2.04, indicating massive relative and even absolute depletion of the heavy rare earth elements simultaneous with an approximately four-fold increase in the light REE.

Light REE depletion in the more primitive looking, potassium and titanium-poor basalts from the southern part of the ridge may be ascribed either to clinopyroxene fractionation, or better to derivation from the partial melting of a garnet-harzburgite source rock, which would represent a partially residual mantle^{4,7} after the prior elimination of clinopyroxene from the assemblage. Only eclogite fractionation from such liquids appears capable of producing residual liquids with the extraordinary depletion of heavy REE, simultaneous with massive enrichment of other incompatible elements which the more northerly basalts display.

I now suggest that all these basalt magmas erupted between 60° N and 65° N on the mid-Atlantic ridge may be derived from a similar parental magma. The differences arise because the outflow of magma was persistently greater in the area of Iceland itself¹⁰ leading to a greater elevation of the superstructure above the ocean floor. Magmas rising through this thicker superstructure exert a higher hydrostatic pressure at the source region and take much longer periods between formation and the eruption of some of the magmas on the surface, allowing greater scope for fractional crystallization at all pressures during ascent. The remarkable abundance of acid differentiates in Iceland fits naturally into this model.

The relative enrichment of incompatible elements, such as K, P, Y, implies that those basalts erupted at the highest levels (on Iceland itself) represent the residual liquid after the crystallization as olivine-gabbros or eclogites of at least 75% of some parental liquid if fractional crystallization is the sole cause of the variations.

The ultimate source of the primary magmas might be postulated to be the base of the upper mantle, which is the most likely region for an intersection of the geotherm with the solidus. Such a process, which requires a substantial heat flow from the lower mantle, has side effects capable of explaining plate decoupling, ocean floor spreading, "convective" return within the upper mantle and the geochemical problems of magmatic provinces¹¹. It does not require the extensive flow of solid mantle which is required by the "plume" or simple convection hypothesis, but I concede that it is no more successful than any other interpretation at explaining why Iceland should be a point of enhanced magma flow.

The grounds on which Schilling¹ rejected fractional crystallization as the origin of the geochemical differences between the abyssal and subaerial lavas were the increased $^{87}Sr/^{86}Sr$ and $^{207}Pb/^{206}Pb$ values in those basalts enriched in incompatible elements. The assumption that these isotopes cannot be fractionated is implicit. In the face of the evidence for substantial low and high pressure fractional crystallization of these basalts, it may be necessary to re-examine the proposition that isotope ratios do not change during such a process. At least part of the geochemical differences commented on by Schilling¹ are due to low pressure fractional crystallization. Only after these effects have been carefully unravelled will it be possible to determine whether or not there is any residual evidence for mixing of magmas from two fundamentally different mantle sources, one of which might then be interpreted as a mantle plume.

M. J. O'HARA

Grant Institute of Geology,
Edinburgh EH9 3JW

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Wave-like Disturbances in the Ionosphere

BEER¹ has recently suggested that atmospheric gravity waves could be expected to exist in the ionosphere as a result of the supersonic motion of the terminator. He reached this conclusion by drawing an analogy between the proposed production of gravity waves by the supersonic motion of the Moon's shadow on the Earth's atmosphere during a solar eclipse², and the supersonic motion of the Earth's terminator. At the mesopause the extent of the terminator's supersonic motion can be as great as $\pm 55^\circ$ latitude.

We find Beer's suggestion particularly interesting in the light of our observations of the behaviour of ionosphere electron temperature and density measured by the Langmuir

probe experiment on ESRO-1A (refs. 3 and 4). During the lifetime of the satellite the rotation of the orbital plane with respect to the Earth-Sun line, and the precession of the position of perigee in the orbital plane, meant that a sweep of local times was obtained at all latitudes and over a range of altitudes. Plots of electron temperature and density against UT were prepared for each orbit and visual inspection of these plots revealed on occasions the wave-like disturbances first reported by Raitt⁵, and described here. Careful checking of satellite "house-keeping" data indicated that the observed variations were of geophysical origin, and were not due to equipment malfunction or varying satellite attitude.

Useful data were retrieved from 2,200 of the first 2,800 orbits of ESRO-1A, and these data were searched for the occurrence of wave-like disturbances. Evidence of such disturbances was found on 120 orbits. All 120 examples occurred after the satellite entered the dawn sector and were usually restricted to the latitude range $\pm 60^\circ$.

An example of the effect is shown in Fig. 1 which shows the variation of electron temperature around a complete orbit. The electron density always showed a similar variation, although with a rather complex phase relationship that could not be clearly established. For illustration purposes we have used the linear scale temperature plot because the wave-like nature of the disturbance is more pronounced than in the logarithmic density plots. (An additional feature of Fig. 1 is the occurrence of "sub-auroral temperature peaks"—narrow bands of greatly enhanced temperature occurring at conjugate latitudes between about 45° and 55° during times of magnetic disturbance⁶.)

The waves detected by ESRO-1A first became evident in early February 1969 when the local solar times for the satellite equator crossings were 0700 and 1900. They were still evident in late April, when the equator crossing times were 0500 and 1700; that is, the waves appeared to be restricted to a band within 2 h (30° longitude) of the terminator. The example of Fig. 1 shows waves only in the dawn sector; although on a few occasions of spectacular disturbance the waves also extended into the dusk sector where they were of very much smaller amplitude. Because of the configuration of the orbit it was impossible to determine whether this small amplitude revealed a true difference in dawn and dusk behaviour, or an altitude dependence.

If the horizontal wave velocity is very much smaller than the satellite velocity, then a "wavelength" estimate may be obtained by multiplying the time between successive maxima by the horizontal component of satellite velocity. For the 120 examples detected by ESRO-1A, this resulted in a distribution of "wavelengths" which peaked near 1,000 km.

The fluctuations we observe may be magneto-hydrodynamic waves stimulated by gravity waves in the lower ionosphere causing plasma motion parallel to the magnetic field, but able to propagate only in those regions of the ionosphere where Lundquist's criterion $BL\sigma\rho^{-1} \gg 1$ is valid, where B =magnetic field, L =characteristic size of plasma, σ =conductivity of plasma, ρ =density of plasma.

This would go some way to explaining the asymmetry in our results between dawn and dusk for which the orbit configuration is such that the satellite is generally at higher altitudes in the dawn sector than the dusk sector; also even at similar heights the plasma conditions at dawn are quite different than at dusk. In fact both situations tend to satisfy the Lundquist condition since in the dusk sector we have a dense (O^+) ionosphere of lower conductivity than the high less dense dawn sector of (H^+/H_e^+) ionosphere of higher conductivity.

Because of the restricted times (within a few hours of the terminator position) and locations (within the latitude range $\pm 60^\circ$) of the ESRO-1A observations of wave-like disturbances, we put forward our observations as possible evidence for Beer's hypothesis that gravity waves could be generated at the terminator longitude and would be expected

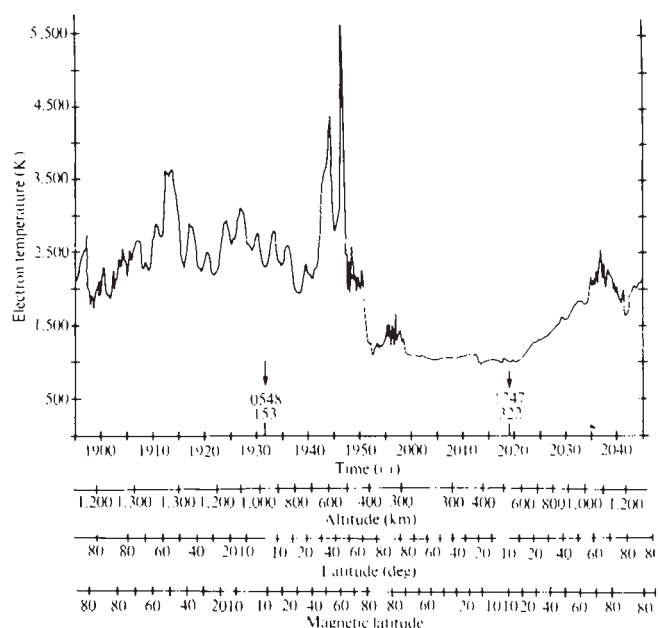


Fig. 1 ESRO-1A electron temperature data. Orbit 2,604, April 5, 1969. Arrows ($\downarrow$) mark LST of equator crossing (top) and longitude of equator crossing (bottom).

to produce ionospheric effects as a result of the supersonic motion of the terminator.

W. J. RAITT

Mullard Space Science Laboratory,
University College, London

D. H. CLARK

School of Physics,
University of Sydney

Received April 2, 1973.

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Phosphate in Sediments off South-western Africa

RECENT work¹ has shown that the unconsolidated sediments of the continental shelf off south-western Africa are considerably enriched in phosphate compared with the values considered usual for clastic sediments ($<0.2\% P_2O_5$), and it is interesting that phosphorite still appears to be forming on a significant scale in the diatomaceous muds off south-west Africa^{2,3}. This may be the first well documented occurrence of modern phosphorite formation.

As part of a general geological survey we have determined the distribution of phosphate in 900 samples collected on a 10-mile grid extending from the coast to depths of 1,500–2,000 m (Fig. 1), allowing us to present a more detailed picture than hitherto possible of the distribution of phosphate and to provide a regional synthesis of the origin of the deposits. The phosphate determinations were made colorimetrically by the Phosphate Development Corporation, Phalaborwa, Transvaal.

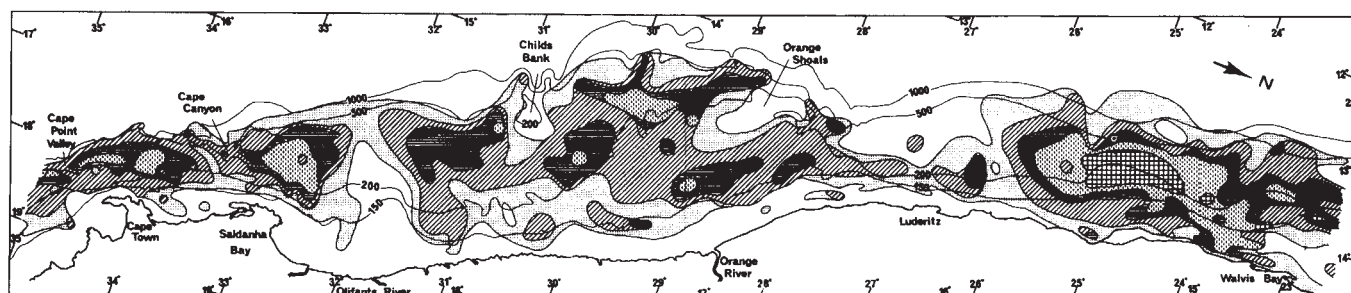
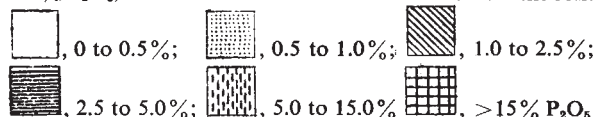


Fig. 1 The distribution of phosphate (as % P_2O_5) in unconsolidated surface sediments from the continental margin of south-western Africa.



The bathymetry is based on soundings by the Marine Geology Group, University of Cape Town, and depths are in m.

We find that the phosphatic sediments (>0.5% P_2O_5) are concentrated on the outer continental shelf (150–500 m) and that they differ in character to the north and south of Lüderitz. To the south they contain, on average, 2% P_2O_5 and at most 9% P_2O_5 . The sediments richest in phosphate (>2.5% P_2O_5) are concentrated on the outer shelf (200–500 m), except between Lüderitz and the Orange River where the concentration is chiefly on the middle shelf (150–250 m). The sediments of the inner shelf (<150 m) and those of the slope (>500 m) are not commonly phosphatic, except south of 32° S where phosphatic sediment is found as deep as 1,000 m.

The phosphatic sediments of this southern area are chiefly sands and muddy sands, some of which are gravelly and many of which are glauconitic, the glauconite concentration reaching 90% (ref. 4). This mineral is associated with subangular, sand-sized fragments of phosphorite, and may itself be partially phosphatized⁵. The fragments of phosphorite are identical to the large slabs of this rock commonly dredged from the middle and outer shelf in this region⁶. These slabs are assumed to be fragments of Eocene to Miocene bedrock, much of which is glauconitic^{7,8}. We consider that the phosphatic and glauconitic sediment associated with the phosphorite is probably a residual or lag-type placer deposit mantling a downward warped late Tertiary erosion surface^{4,5}. The preservation, at surface, of these largely relict sediments is due to the very slow rate of late Tertiary and Quaternary sedimentation prevailing over the outer shelf. Where this rate increases, as on the inner shelf and on the slope, the relict phosphorite is either buried or diluted.

Low phosphate concentrations on the outer shelf reflect slight variations in the sedimentation pattern. For example, off Lüderitz it seems that highly quartzose sediment from the Orange River was widely distributed during low sea level times, thus burying or diluting residual phosphatic sediment⁹. On the Orange Shoals and the Childs Bank, Quaternary carbonate sedimentation has been slightly higher than on the deeper surrounding seafloor and the relict phosphatic components of the sediment on these rises has been diluted correspondingly. By contrast, the low phosphate content of the sediment off the Olifants River is due to burial of the downward warped Tertiary erosion surface by a prograding wedge of terrigenous muds. The phosphate depletion in the sediments of the Cape Canyon and Cape Point Valley, which cut through Neogene and Palaeogene strata and locally exposed Cretaceous rocks^{7,8}, may reflect their derivation from older (Palaeogene or Cretaceous) and less phosphatic outcrops than those beneath the middle and outer shelf. An isolated sample of phosphatic sediment (5.4% P_2O_5) from the canyon floor represents transported material.

North of Lüderitz, phosphatic sediment (>0.5% P_2O_5) covers much of the inner shelf and uppermost slope in addition to the outer shelf. The phosphatic sediment of this northern area contains, on average, 4% P_2O_5 and, at most, 22% P_2O_5 . Near Walvis Bay, diatomaceous mud is widespread in the inner

shelf (50–150 m). Our samples of this mud usually contain <0.5% P_2O_5 both at surface and at depth within sediment cores up to 1 m long, and the distribution of the mud is clearly reflected by that of phosphate-poor sediment (see Fig. 1). Richly phosphatic sands, which may be gravelly or muddy but are not normally glauconitic, occur on the outer shelf seawards of the ooze. The phosphate of the sands is concentrated in well-rounded, dark brown (ferruginous and manganiferous), sand-sized pellets (with median diameter of 0.1–0.5 mm) of more or less pure phosphorite. The phosphate mineral is francolite, and the pellets contain about 32% P_2O_5 . One mechanism of pellet formation has been proposed by Soviet geologists^{1–3} who found, scattered within the diatomaceous muds, some soft, friable, sand and gravel-sized clots of phosphatized sediment. They suggested that these clots would lithify with further diagenesis and that subsequent mechanical reworking of the mud during changes of sea level would give rise to concentrations of pellets and small nodules of phosphorite. Some of the pelletal deposits so formed have been subsequently lithified in the past to form pelletal conglomeratic phosphorite. We have dredged angular fragments of this rock type, containing up to 20% P_2O_5 , from the centre of the zone of richly phosphatic pelletal sediment between Lüderitz and Walvis Bay. As some of the pellets in the overlying sediment are clearly composite, erosion of this outcropping phosphorite must also have been a source of pellets. The relation between the apparently modern phosphorite of the ooze belt, the unconsolidated phosphatic pelletal sands of the outer shelf, and the pelletal conglomeratic phosphorite is evidently very complex and a detailed coring and dredging programme is required to resolve it.

Both to the north and south of Lüderitz many of the largely terrigenous fine-grained sediments of the inner shelf and upper slope are very slightly phosphatic (0.2–0.5% P_2O_5). These same sediments are relatively rich in organic matter (>1% C_{org}) and contain abundant faecal pellets. The enrichment of the sediments in organic matter seems to have given rise to conditions favourable to the diagenetic formation of small amounts of phosphate minerals in such microenvironments as faecal pellets and foram tests^{1,5}. The main exceptions to this pattern are on the inner shelf off the Orange River, where the rate of sedimentation of terrigenous detritus is very high, and on the lower parts of the slope at depths where organic matter is very scarce because of its solution in the water column¹⁰. The only other major source of phosphate is fish debris, which was mainly concentrated, although in very small amounts, in the relict sediment of the outer shelf¹.

The abundance of phosphatic sediment on this shelf is indirectly due to the upwelling of nutrient enriched oceanic waters and consequent high biological productivity. Modern phosphorite seems to be forming diagenetically in the fine-grained organic rich sediment typical of this area of upwelling. But it is only forming in very small amounts. The bulk of the

phosphate is bound in relict detrital gains of phosphorite. These were derived in the late Tertiary, or during low sea level times in the Pleistocene, by mechanical reworking of diatomaceous muds (north of Walvis Bay) or by the erosion of previously existing phosphorite (throughout the area). The lack of widespread modern phosphorite formation signifies a wide difference in environmental conditions between the present time and the early to middle Tertiary (when phosphorite formation predominated locally).

The rich relict phosphate deposit near Walvis Bay is probably about 0.5 m thick and may constitute a reserve of some 4×10^9 t P_2O_5 . The grade of the deposit could be benefited by screening and its potential lies in its situation 700 miles nearer to Walvis Bay than the nearest land deposits of comparable grade at Saldanha Bay. As the phosphatic fraction of the sediments south of Lüderitz is not likely to be $>16\%$ P_2O_5 , the average for local source rocks⁶, the southern deposits have no immediate economic interest.

C. P. SUMMERHAYES

Woods Hole Oceanographic Institution

G. F. BIRCH

J. ROGERS

Marine Geology Unit,
University of Cape Town

R. V. DINGLE

University of Aberdeen

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Tidal Resonance in the Coral Sea

THE "age of the tide" is a measure of the time lag between new or full Moon and the following spring tide. In the Coral Sea, the age of the tide is negative¹. This behaviour is rare in the ocean. In terms of the tidal response function this means that there is a negative phase change in the $\bar{R}_2^2(\omega)$ response function², between the frequencies of the dominant M_2 and S_2 constituents.

Suspecting that this may be the consequence of a resonance, I have tried fitting a response function of the form

$$\bar{R}_2^2(\omega) = A + B\omega + C/(\omega - D)$$

to the harmonic constants for Cairns³. The first two terms correspond to the background and the third term to the resonance. Only two terms were used for the background to reduce the number of free variables. I estimated the complex variables A to D by minimizing the expression

$$\sum_j (h_j - \bar{R}_2^2(\omega_j)e_j)^2$$

where h_j is the complex harmonic constant relative to Greenwich, for the semi-diurnal constituent with angular velocity ω_j and equilibrium amplitude e_j (ref. 4).

The function obtained in this manner is shown in Fig. 1. The data points are the values of the response functions at the

twelve constituent frequencies calculated from the relation,

$$\bar{R}_2^2(\omega_j) = h_j/e_j$$

The error bars indicate the expected standard deviation due to noise in the original record⁵. For the constituents N_2 , M_2 , S_2 and K_2 the s.d. is less than the radius at the data points.

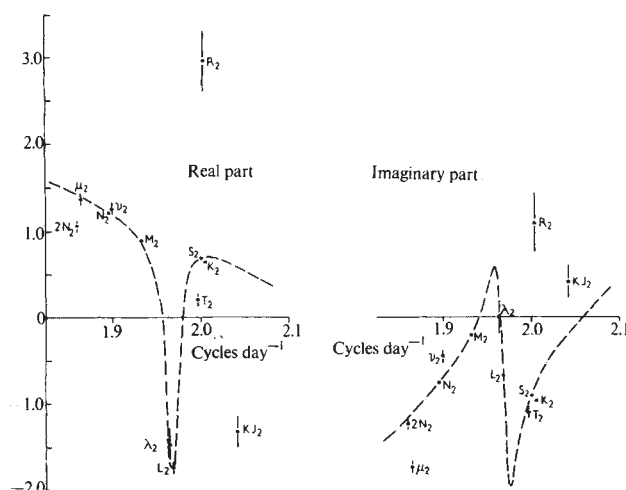


Fig. 1 Real and imaginary part of the $\bar{R}_2^2(\omega)$ tidal response function at Cairns ($16^\circ 55' S$, $145^\circ 47' E$), showing the influence of a resonance in the Coral Sea.

The coordinate of the resonance is $1.969 - i 0.009$ cycles day⁻¹ with an expected s.d. in both coordinates of 0.003. The imaginary part corresponds to a decay time of 18 days.

Initially it also seemed possible that the large phase differences might be due to the radiational tide^{2,6}, but this would have meant that the radiational component of S_2 was much larger than its normal value of about 20% of the gravitational component. Further, the data of Fig. 1 show that the resonance hypothesis is supported by the behaviour of the L_2 and λ_2 constituents, which would not be affected by the radiational tide.

The existence of the resonance is also confirmed by calculations using harmonic constants for Cooktown³ and Port Moresby⁷. But in these cases the fit to the harmonic constants is not as good as it is by Cairns. Non-linear interactions may, however, introduce an error of up to 25% in the L_2 constituent at Cairns.

If the resonance is an oceanic resonance and is not due to the movement of nearby land masses^{8,9}, then I believe this to be the first time an oceanic resonance has been observed lying within a tidal band. A fuller discussion of the method and of its errors will be published later.

D. J. WEBB

CSIRO Division of Fisheries and Oceanography,
Cronulla, NSW

Received January 30, 1973.

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Origin of Bright Spots in High Resolution Dark Field Electron Microscopy

SMALL bright spots (0.3–0.7 nm) have been observed in the dark field images of evaporated carbon films¹ but their origin has not been explained. Here I suggest that these bright spots are present in the dark field image at an overfocus (a higher objective lens current) of $300 \text{ nm} \pm 50 \text{ nm}$ relative to the bright field focus (the finest granulation in the phase structure).

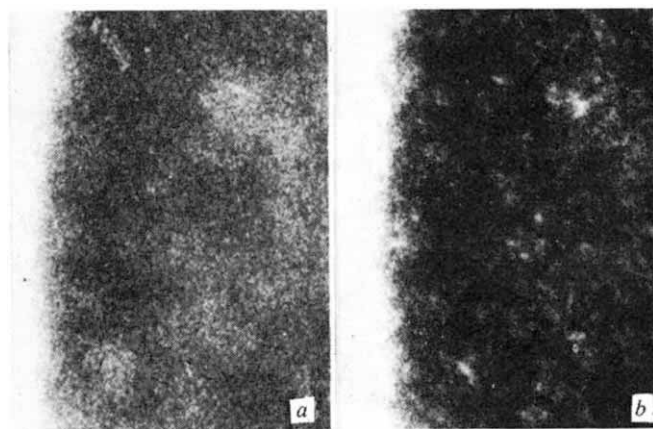


Fig. 1 *a*, Dark field image of a thin ($3.5 \text{ nm} \pm 30\%$) carbon film taken at the objective lens current for bright field focus ($\Delta f = 0$). *b*, Bright field spots at maximum sharpness at an overfocus of $\Delta f = \pm 300 \text{ nm}$ in respect to the bright field focus. Film magnification 95,000, final magnification 2.1×10^6 .

According to Thon², there is then an image of a plane in the bright field which is slightly overfocused in relation to the geometrical or Gaussian image plane. For an electron microscope with a spherical aberration coefficient of 1.6 nm, such as the Philips EM300, this overfocus is $50 \text{ nm} \pm 30 \text{ nm}$. Bright spots therefore occur in the dark field image at an overfocus of $1.6 \text{ nm} \pm 80 \text{ nm}$ in relation to the geometrical image plane. The dark field images of a carbon foil, obtained using conical dark field illumination³, are shown at both focus conditions in Fig. 1. To contribute to the dark field image, the electrons were scattered through angles between α 0.0001 and 0.001 rad.

To account for the small size of the bright spots, so far unexplained, and for their observation at overfocus with respect to the bright field focus, a model is proposed based on a wave interference phenomenon well known from classical optics⁴. A plane wave passing a circular aperture of diameter a generates, at a point P behind the aperture, through constructive interference, a maximum with an intensity of four times the original intensity. The location of P is determined by the condition that the distance between P and the rim of the aperture exceeds the distance to the centre by half a wavelength. For the distance of P to the aperture (b), the relation $b = a^2 (4\lambda)^{-1}$ can be calculated (λ = wavelength). From the location of the first dark ring in the interference pattern around P, one can show that the diameter at half intensity of the high intensity spot at P is somewhat less than one-third of the aperture diameter a . I think that a similar process produces the dark field bright spots, the wave fronts being generated by Bragg reflexion of electrons on small crystals present in the carbon film.

To substantiate this, the following points can be made.

First, inherent in the concept of Bragg reflexion is the idea that the outgoing wave from the crystal is a plane

wave over the crystal area and therefore not very different from a plane wave passing an aperture.

Second, Ban *et al.*⁵ have found indications that small graphite like crystals are present in carbon foils. Crystals with lattice spacings from 0.35 nm or greater (including graphite) can lead to Bragg reflexion inside the acceptance angle α of the dark field geometry. In general the "apertures" for the wave interference, formed by the crystals, will not be exactly circular and the high intensity maxima will occur at a distance corresponding to some average diameter of the crystals.

Third, the bright points are found at overfocus in relation to the geometrical focus. Relative to the direction of travel of the electrons, an image of a plane occurs behind the object, just where, according to the present model, one would expect the interference maxima.

Fourth, putting b equal to the defocus distance of $350 \text{ nm} \pm 80 \text{ nm}$, the dimensions of the crystals involved in the Bragg reflexion can be calculated from the formula $b = a^2 (4\lambda)^{-1}$. For 80 keV electrons we have $\lambda = 4.2 \text{ pm}$ (picometre) and, for the diameter a of the crystals, values in a range of 2–2.8 nm are found. One would expect the size of the high intensity interference maxima (the bright spots) to be somewhat less than one-third of this crystal size, about 0.5–0.7 nm. Smaller spots can be produced by photographic printing procedures. This was seen in photographs of a light optical analogue of the interference phenomenon. The observed size of the bright spots, 0.3–0.7 nm, is therefore in agreement with the spot size expected from the interference process, and thus constitutes an independent argument for this physical mechanism causing the dark field bright spots.

A full account containing bright and dark field through focus series will be published elsewhere, together with a consideration of the implications of the model for the dark field images on biological material in the electron microscope.

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G. J. BRAKENHOFF

Laboratory of Electron Microscopy,
University of Amsterdam,
14, Plantage Muidergracht, Amsterdam-C

Received February 15; revised March 29, 1973.

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Magnetic Fields, Ball Lightning and Campanology

WOODING suggests¹ that ball lightning is a plasma vortex ring structure produced by a process similar to the ablation of a solid surface by a high power laser pulse. A plasma vortex ring structure requires a magnetic field; here I present two pieces of evidence to show that a magnetic field is associated with ball lightning, and which may help to confirm this hypothesis.

Mühleisen² quotes a story of the disturbance of a ship's compass when a lightning ball was dancing about in the rigging; and the following story³ would seem to indicate the presence of quite a powerful magnetic field.

"In the parish of Samford-Courtney in Devon on October 7, 1811, a sudden darkness came on, and a fire ball fell in the vicinity of the church. The ringers in the belfry, ringing at the time, declared that they never knew the bells go so heavy, and were obliged to desist ringing. Looking down from the belfry into the church, they perceived four fire balls, which suddenly burst, and the church was filled with fire and smoke, some of which ascended to the tower, where a large beam, on which one of the bells was hung, was broken, and the gudgeon breaking, the bell fell to the floor."

In England it is usual to ring the church bells in changes, each bell swinging through 360°, alternately clockwise and anticlockwise, at each pull of the rope; a speed of about twenty-five changes a minute is usual. The magnetic field strength necessary to stop the ringing of bells may be calculated as follows.

The potential generated when a disk of area A rotates at N revolutions s^{-1} in a magnetic field of strength H gauss is $NHA \times 10^{-8}$ V, $=8.85 \times 10^{-5} H$ V, when $N=0.5$ and $A=17,700$ cm², the radius from the line of gudgeons to the lip being taken as 75 cm for a bell of average size (15 cwt). The energy dissipated when an electric current flows in a conductor is $V^2 t/R$ W s. Setting $t=2$ for one revolution of the bell (one pull of the rope).

Work done at each pull $= (8.85 \times 10^{-5})^2 H^2 2/R = 100$ assuming that a ringer must stand when he must apply a force equivalent to more than 10 kg weight through 1 m or, say, 100 W s, at each pull to get the bell up to the balance. The generated potential will, I assume, produce eddy currents which flow against the resistance R . The resistance will be taken as that of the cross-section of the bell wall over a length of half the average circumference, $=3.5 \times 10^{-6} \Omega$ when average diameter = 70 cm, average wall thickness = 7 cm, length of wall from lip to crown staple = 80 cm, and specific resistance of bronze $=18 \times 10^{-6} \Omega$ cm. For these numerical values, $H=150$ gauss.

This calculation can only be taken as an estimate because, first, the bell is not a rotating disk, but a compound pendulum consisting of a hollow vessel swinging through 360° although average angular velocity $=0.5$ revolution s^{-1} , and, second, the resistance against which the eddy currents flow cannot be accurately calculated, as one does not know how the currents flow within the bell.

A. J. F. BLAIR

5170 Jülich,
Robert Koch Strasse 5,
Germany

Received December 11, 1972

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HNCO in the Galactic Centre

THE $1_{01}-0_{00}$ transition of the HNCO molecule at 21.9817 GHz has been detected using a 1.4 cm parametric amplifier at the 36 foot telescope of the US National Radio Astronomy Observatory¹. The present observations were made with the same receiver and the 85-foot Maryland Point telescope of the US Naval Research Laboratory. The receiver has a single sideband system temperature of 600 K achieved by the use of a degenerate parametric amplifier with 20 dB of gain².

The filter bank receiver used to obtain the spectral line data consisted of 20 channels each 0.5 MHz in width giving a total velocity span of 135 km s^{-1} . At 1.4 cm the beamwidth of the telescope is approximately 2.1 arc min and the beam efficiency is 60%.

A map of the region around the galactic centre source Sgr B2 was made (Fig. 1). The spacing of the points was 2.0 arc min. The peak antenna temperature observed was 0.4 K and line velocity was 60 km s^{-1} . The shape of the contours is influenced by the sampling points which were taken east-west and north-south of the position of greatest line intensity.

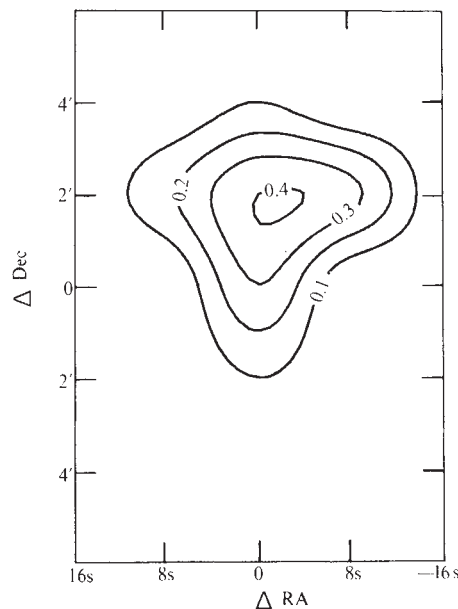


Fig. 1 Contour map of the distribution of HNCO in the galactic centre source Sgr B2. The offsets in RA and dec are from the central position RA (1950) = 17 h 44 min 11 s and dec (1950) = $-28^{\circ} 22' 30''$. The contour levels are antenna temperature.

The HNCO cloud appears to have a minimum diameter of ~ 5 arc min and is centred ~ 2 arc min north of the H_2O source Sgr B2(OH). A similar northern emission peak was found from mapping observations of both the $4_{04}-3_{03}$ line of HNCO at 87.9 GHz (ref. 3) and the 1_0-0_0 lines of CH_3OH at 48.3 GHz (ref. 4). The maximum intensity of the (3,3) line of NH_3 (ref. 5) and the highest optical depth of the $1_{11}-1_{10}$ line of H_2CO (ref. 6) are also observed in the direction of the HNCO emission peak. Thus this position should be searched for other molecular species to learn if it might be the principal region for molecular formation in Sgr B2 and hence of greater chemical interest than the well known Sgr B2(OH) position.

At a distance of ~ 10 kpc, the HNCO cloud has a diameter of ~ 15 pc. If we adopt 5×10^{14} cm⁻² as the total projected density of HNCO (ref. 3), the total mass of HNCO is $\sim 0.02 M_{\odot}$. The molecular hydrogen density in the HNCO cloud is $N(H_2) \geq 1.8 \times 10^5$ cm⁻³ (ref. 3); thus the abundance of HNCO relative to H_2 is $N(HNCO)/N(H_2) \leq 6 \times 10^{-11}$ which agrees surprisingly well with $\sim 2 \times 10^{-11}$ which would be expected from the product of the cosmic abundances of the constituent atoms.

We acknowledge the help of the telescope operators and W. Waltman for maintaining the receiver. In making the observations and reducing the data we were assisted by J. Jafolla, F. O. Clark, P. Giguere and T. Davis. L. E. Snyder acknowledges partial support from a US National Science Foundation research grant. The US National Radio Astron-

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DAVID BUHL

National Radio Astronomy Observatory,
Green Bank, West Virginia

LEWIS E. SNYDER

University of Virginia,
Charlottesville, Virginia

PHILIP R. SCHWARTZ

E. O. Hulburt Center for Space Research,
Naval Research Laboratory,
Washington, DC

JOCHEN EDRICH

University of Denver,
Denver, Colorado

Received May 21, 1973.

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Magnetoresistive Amplifier

THE construction of a bistable circuit with two magnetoresistors¹ raises expectations about other applications to active electronic circuits. The bistable circuit has some resemblance to valve and transistor circuits, but they are capable of acting as single element bistable multivibrators also; moreover, a single active element suffices for amplifiers and oscillators. Is the magnetoresistor equally versatile?

For a magnetic field B well below saturation, the resistance R is increased above an initial R_0 according to a square law

$$R = R_0 + aB^2 \quad (1)$$

If the current in the magnetoresistor is made to control its own magnetic field², rather than that of another, there is simply an increase in resistance, which is steep for an efficient magnetic circuit. A negative slope in the voltage/current characteristic is no longer possible at first sight.

But if a constant initial magnetic field is superimposed, the self magnetic field of the resistor can be directed against this initial bias. As the current now increases, resistance falls and the rate of fall can become so large that voltage/current curves slope negatively over part of their range.

$$R = R_0 + c(I_B - I)^2 \quad (2)$$

where the constant c includes a and the constants of the magnetic circuit; I_B is a bias current in a winding with the same number of turns as the winding which passes I through the magnetoresistor. If R_0 includes the resistance of that winding and V is the potential difference across the combination, then

$$V = IR_0 + cII_B^2 - 2cI^2I_B + cI^3 \quad (3)$$

$$dV/dI = R_0 + cI_B^2 - 4cII_B + 3cI^2 \quad (4)$$

The condition for the occurrence of negative slope is found by equating (4) to zero and solving the quadratic; it is

$$R_0 \leq (c/3)I_B^2 \quad (5)$$

In Fig. 1A I show a magnetoresistor controlled jointly by a series winding and a separate bias coil. Figure 1B is a family of curves computed from equation (3) with bias field as parameter; Fig. 2 gives experimental negative resistance curves

with the same equipment as the bistable circuit¹, except that four 500 Ω magnetoresistors are connected in series/parallel to act like a single 500 Ω element with a four-fold power rating. Depending on the choice of load line and external components, the element operates continuously at room temperature as an amplifier or an oscillator or a trigger circuit.

As an example of a d.c. amplifier, an operating point $V_{cd} = 20$ V, $I_c = 43$ mA is chosen with load at 210 Ω ; in Fig. 1A terminals c and d are the output pair connected to a voltage source through the load resistance. Bias does not in fact require a separate winding, but is provided by passing a constant current (of 60 mA) from e to c . The winding ab is released to become the input pair of terminals. A power gain of 75 is recorded. The input may be imagined to create a small shift in the bias, which has a large effect on the operating point.

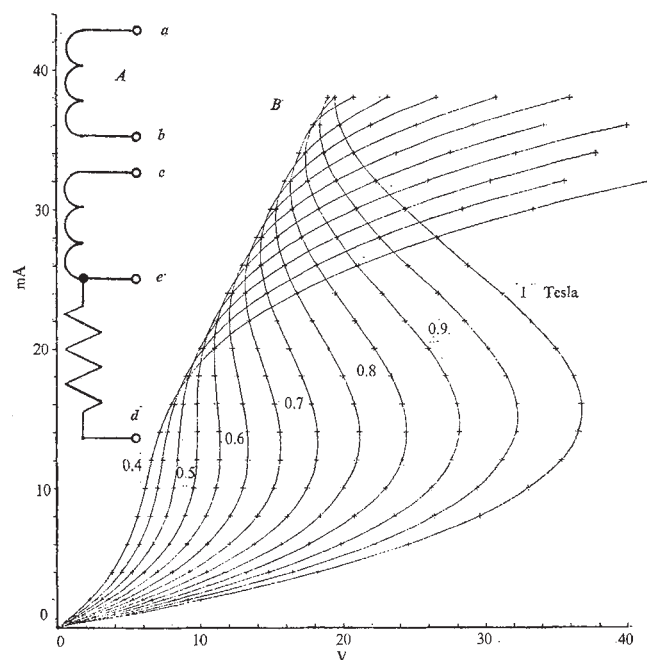


Fig. 1 A, Magnetoresistor with series control coil and separate bias coil. B, Current/voltage characteristics with bias field as parameter. (Computer graph plot. I_c against V_{cd} .)

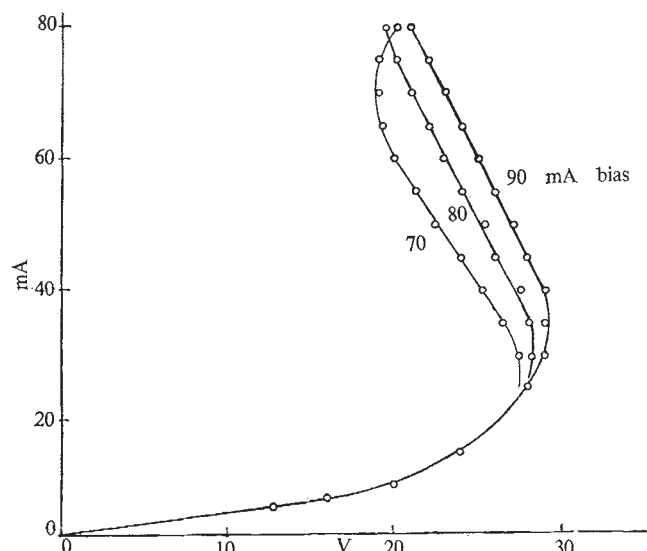


Fig. 2 Experimental current/voltage characteristics for a biased self-controlled magnetoresistor

An efficiency of 12% is measured, but if the bias had been provided by a permanent magnet 21% is indicated.

For an oscillator, a capacitor is connected between *c* and *d*, the bias is applied to *a* and *b* through chokes, and the power for the oscillator is supplied to *c* and *d* again through chokes, which have limited frequencies to 600 Hz so far. But if the magnetoresistor is a Corbino disk with no external circuit, natural frequencies of oscillation are observed at 400 MHz (ref. 2). The present theory is believed to apply to this example of self-excitation but there need be no series coil to augment the self-magnetic field of the disk³.

The biased self-controlled magnetoresistor has interesting characteristics, which imply applications of wide versatility. The magnetoresistive effect is fundamental and does not wholly depend on the technology of high purity, single crystal semiconductors.

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D. MIDGLEY

Department of Electronic Engineering,
University of Hull

Received April 27, 1973.

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BIOLOGICAL SCIENCES

DNA Differences between Flax Genotrophs

CERTAIN environmental conditions can induce genetic changes in particular varieties of plants¹⁻⁵; these changes are stable and inherited over many generations. One of the best examples of this involves the flax variety Stormont Cirrus, which when supplied with certain fertilizers and grown for at least 5 weeks in a heated greenhouse can change from its original form (P1) into a large stable genotroph (L),

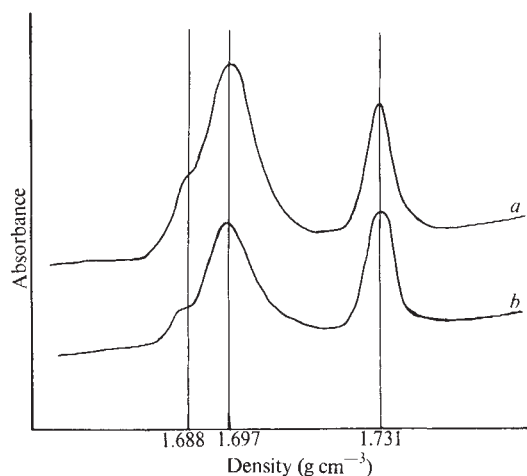


Fig. 1 Caesium chloride equilibrium gradient run for 24 h at 44,770 r.p.m. at 25°C on a Spinco Model E analytical centrifuge. *a*, 2 µg of DNA from L genotroph with a principal band of density 1.697 g cm⁻³ and a secondary band of density 1.688 g cm⁻³. *b*, 1.5 µg of DNA from the S genotroph with a principal band of density 1.697 g cm⁻³ and a secondary band of density 1.688 g cm⁻³. Both samples had 1 µg of DNA from *Streptomyces coelicolor*, density 1.731 g cm⁻³ added as a marker.

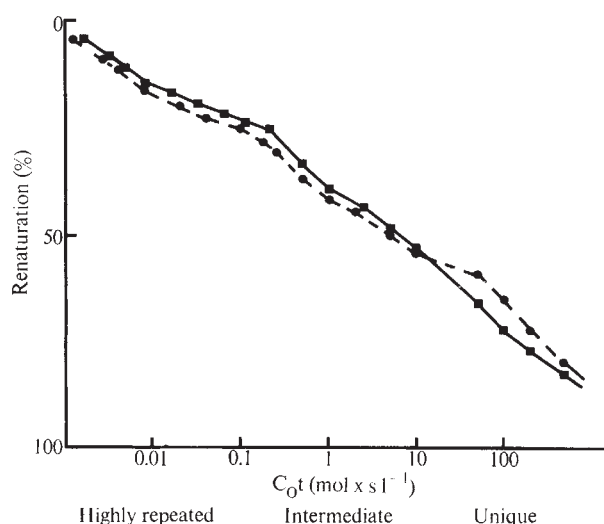


Fig. 2 C_0t curves of DNA from the L genotroph (■—■) and the S genotroph (●- - ●). The DNA was sheared by sonication to a molecular weight of 7×10^5 and incubated at 50 µg ml⁻¹ or 500 µg ml⁻¹ in 0.12 M phosphate buffer (equimolar sodium dihydrogen phosphate and disodium hydrogen phosphate) at 60°C. Single and double stranded material was separated on a hydroxyapatite column also maintained at 60°C. The degree of renaturation was plotted against the C_0t value (ref. 8).

or a small stable genotroph (S), depending on the fertilizers supplied^{1,6}. L plants may be up to six times the weight of S plants, depending on the environment in which they are grown, with the P1 type having a weight in between those of L and S.

L and S behave as two distinct genetic types, giving equilinear inheritance when they are reciprocally crossed, and no transmission through reciprocal grafts¹. L has been shown to have 16% more nuclear DNA than S as shown by Feulgen staining⁶. The P1 type has a DNA content intermediate between that of L and S, having 10% less DNA than L but 6% more DNA than S.

I have now characterized the nature of the DNA differences between the genotrophs, with the ultimate aim of attempting to elucidate the nature and mechanism of induction of the changes in DNA and in the phenotype. The method of extraction of DNA from the L and S genotrophs will be described elsewhere (my unpublished work). The extracted DNA was analysed by ultracentrifugation and renaturation. The DNA from both types formed two bands in neutral caesium chloride gradients (Fig. 1). The principal band had a density of 1.697 g cm⁻³ and a secondary band a density of 1.688 g cm⁻³. The amount of DNA contained in the satellite band was approximately 15% and was about the same in samples from both types. The densities of both the satellite and the main band increased by 0.01 g cm⁻³ after heat denaturation.

The renaturation of the DNA was followed optically⁷ and by separation on hydroxyapatite⁸. From the results (Fig. 2) it was apparent that the DNA from both L and S genotrophs contained some sequences which were highly repeated, others which were less repeated and some unique⁸. The highly repeated sequences make up 24% of the total DNA from L and 28.5% of the total in S (my unpublished work). The satellite DNA was included in this fraction. The difference between the proportion found in L and S could be explained as follows: if the extra DNA in L contained none of the highly repeated sequences, then the consequent dilution of such sequences in L would result in the differences observed.

The unique sequences form 45% of the total DNA in S and 40% of the total DNA in L; again this difference is

accountable in terms of the dilution factor mentioned earlier. The complexity of the unique sequences, calculated against a value for *E. coli* of molecular weight 2.8×10^9 (ref. 9), is 2.1×10^{11} ; this agrees well with the value calculated from the total DNA content per nucleus¹⁰ which is 1.97×11 , assuming these unique sequences make up 45% of the total DNA in S and 40% of the total DNA in L.

The intermediate sequences, that is neither highly repeated nor unique, make up the remainder of the DNA which is 26.5% in S and 36% in L. This intermediate fraction in L seems to be composed of two families of sequences: one renaturing at about the same rate as the intermediate sequences in S; the other renaturing more slowly. The extra DNA in L could be in this latter fraction which was not observed in S.

An increase in the concentration of any type of sequence would cause an increase in the rate of renaturation of this sequence. The extra DNA in L was found in the slowest renaturing portion of the intermediate sequences and only the unique sequences renature more slowly than this. Thus the extra sequences must have been derived by increasing some of the unique material. In that case the difference in the rates of renaturation between the additional intermediate and unique material suggests that the extra DNA is present at seventy times the multiplicity of the unique sequences. This means that the extra DNA in L would be equivalent to 0.4% of the unique sequences at a multiplicity of seventy times.

How this increase in DNA is brought about by the fertiliser treatments is still unknown. But specific portions of the genome have been shown to be differentially increased or decreased, and the difference inherited, in other systems: for example in *Drosophila* the number of ribosomal genes at a particular locus can be increased or decreased depending on the chromosomal constitution¹¹. Although this effect is not caused by an external treatment, a mechanism must exist to preferentially increase and stabilize a portion of the genome and in flax something similar may occur with parts of the genome.

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C. A. CULLIS

John Innes Institute,
Colney Lane,
Norwich NOR 70F,
Norfolk

Received February 5, 1973.

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Environmentally-induced Changes in the Brains of Elderly Rats

ANIMALS that have been exposed to environmental stimulation develop marked differences in brain structure and biochemistry when compared with animals that have not been exposed in this way¹⁻⁴. Factors influencing the magnitude of these changes are the duration of rearing and the age of the animals when the environmental separation takes place. Most studies have focused on young animals because the brain is thought to be

more plastic at this stage. As a result, temporal analyses of the induced changes have usually been limited to fairly short periods of environmental exposure early in the animal's life. Rosenzweig *et al.*⁵ have suggested that, in rats differentially reared from weaning, the induced differences in brain weight reach a maximum during the first 30 d of separation, after which they decrease. But the evidence for this biphasic response has been based on relatively short periods of differential rearing (15-160 d).

The aims of our study were two-fold. First, to test whether the early cerebral changes induced in weanling rats could still be detected after longer periods of environmental separation; second, to determine whether the brain remains capable of morphological change with increasing age. Rosenzweig *et al.*⁶ found that the brains of 105-d-old rats were as easily modified as those of younger weanling animals, whereas Riege⁶, using a much longer period before environmental separation (300 d), found evidence of a decreased rate of brain change in response to the differential rearing conditions. This latter study suggested that the potential for morphological change may indeed diminish in older animals.

Twenty litter-mate pairs of Wistar albino rats were matched for body weight and divided into two groups at weaning. The first group was divided equally between two open-mesh cages, both of which were supplied with an assortment of "toys" which were changed daily (enriched condition). Rats from the second group were placed singly into small stainless steel boxes with solid sides (isolated condition). Both groups were kept in their respective environments for 509 d, after which they were sub-divided equally into those animals that were to remain unchanged in their respective environments (enriched and isolated) and those that were to be tested on a Hebb-Williams maze (enriched, tested and isolated, tested).

The behavioural testing period took 21 d, during which time the tested animals were removed from their respective environments for 15-30 min once each day and then returned. During maze testing animals were deprived of water for 23 h for reward purposes. Both non-tested groups were maintained on precisely the same schedule. This short daily interval of behavioural testing and attendant handling therefore constituted the only difference between the tested and non-tested groups.

The life expectancy of these Wistar rats is uncertain but it is probably less than 900 d, so that by the end of the experiment the animals had lived for well over half of their expected life span. At the end of the testing period all rats were weighed and killed and their brains were removed, weighed and stored in 10% formalin.

Whole brain weight was obtained by removing the olfactory lobes anteriorly, and posteriorly by a vertical cut immediately behind the cerebellum. The brain was then sectioned coronally immediately behind the posterior pole of the cerebrum, so providing an anterior component which included all of the forebrain and most of the midbrain (forebrain sample) and a posterior rhombencephalic component which consisted mainly of cerebellum and medulla (hindbrain sample). These samples were weighed. After fixation for 1 month in formalin solution the length (from anterior to posterior pole) and the greatest width of the cerebrum were measured in the forebrain sample using a vernier scale at a magnification of $\times 12.5$. All measurements were performed blind.

The results of the maze testing showed that enriched, tested animals made significantly fewer errors than isolated, tested ones, indicating that the difference in learning ability had been maintained throughout the protracted rearing period. Cerebral changes were analysed by an analysis of variance (ANOVA), with environment and testing as the two primary fixed factors.

Both environment and behavioural testing exerted a significant effect on brain weight (Table 1). The whole brain weights of the enriched animals were significantly heavier than those of the isolated (3.09%; $P < 0.0125$) and this difference was entirely due to the forebrain sample. The hindbrain sample showed a non-significant trend in the reverse direction, thereby making

Table 1 Mean Brain Weights (g) after Differential Rearing for 509 Days and Behavioural Testing for 21 Days

	Whole brain	Forebrain	Hindbrain	Forebrain hindbrain
Isolated	1.778	1.276	0.502	2.552
Enriched	1.860	1.365	0.494	2.767
Isolated, tested	1.855	1.328	0.526	2.534
Enriched, tested	1.886	1.377	0.508	2.714
Isolated against enriched				
% diff.	3.09	5.30	-2.55	7.75
$P <$	0.01	0.002	N.S.	0.002
Non-tested against tested				
% diff.	2.81	2.46	3.75	-1.35
$P <$	0.05	N.S.	N.S.	N.S.

the forebrain:hindbrain ratio a sensitive index for the detection of environmentally induced change. This agrees with previous findings⁴.

Testing also increased whole brain weight (2.81%; $P < 0.05$) but the increase was not as clearly localized in the forebrain sample. None of the four possible testing $\times$ environment interactions were significant for brain weight.

Since brain weight depends partly on body weight³, the effect of the latter was removed by analysis of covariance. This did not significantly alter the primary effects indicated by the ANOVA in Table 1, but it did alter the testing $\times$ environment interactions, making them significant in the case of the whole brain ($P < 0.01$) and forebrain ($P < 0.001$). The interactions for hindbrain and for forebrain/hindbrain remained non-significant. Both significant interactions were in the same direction as that shown for forebrain (Fig. 1), indicating that the effect of testing in increasing either whole brain or forebrain weight was apparent only in the previously isolated animals.

Cerebral dimensions also showed significant changes (Table 2). The cerebral cortices of the enriched subjects were 1.38% longer ($P < 0.025$) than those of their isolated counterparts, and while neither the width measurements nor any of the changes due to behavioural testing were significant, there was a significant testing $\times$ environment interaction ($P < 0.05$) for the derived parameter of cortical length $\times$ cortical width (Fig. 2).

These results agree substantially with previous studies^{4,7} in which cerebral length, but not width, was significantly modified by differential rearing. But the combination of length $\times$ width represented the most sensitive measure of changes in cerebral dimension.

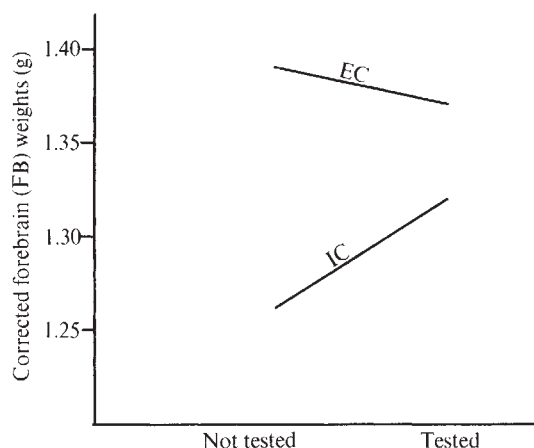


Fig. 1 Testing $\times$ environment interaction on the forebrain weight after a covariate adjustment for body weight. The effect of testing on the enriched animals had no significant effect on forebrain weight (enriched $>$ enriched, tested by 1.25%; N.S.) but testing had a marked effect on the forebrain weight of the previously isolated animals (isolated, tested $>$ isolated by 5.16%; $P < 0.005$). The interaction for the whole brain sample was very similar.

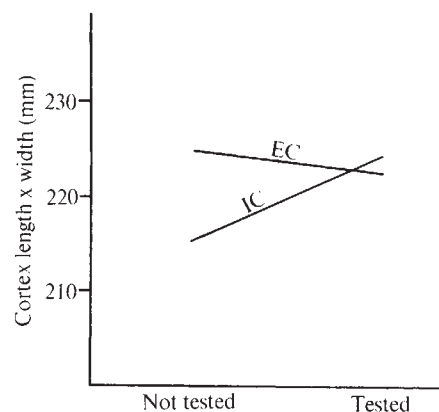


Fig. 2 Testing $\times$ environment interaction on the derived parameter of cerebral length $\times$ cerebral width. Testing had no significant effect on the enriched animals (enriched $>$ enriched, tested by 0.87%; N.S.) but testing had a marked effect on the length $\times$ width of the previously isolated subjects (isolated, tested $>$ isolated by 4.65%; $P < 0.05$).

Analysis of covariance, using body weight as the covariate, did not significantly alter the results obtained by the original ANOVA, but the use of the forebrain weight as the covariate significantly altered the original difference so that enrichment no longer influenced either cerebral length or cerebral length $\times$ width. But the test $\times$ environment interaction on the product of length $\times$ width remained significant as before (enriched $>$ enriched tested by 1.04%; N.S., and isolated, tested $>$ isolated by 3.89%; $P < 0.05$).

Table 2 Mean Cortical Dimensions (mm) after Differential Rearing for 509 Days and Behavioural Testing for 21 Days

	Cortex length	Cortex width	Length $\times$ width
Isolated	14.46	14.87	214.64
Enriched	14.87	15.19	225.85
Isolated, tested	14.73	15.25	224.63
Enriched, tested	14.73	15.19	223.87
Isolated against enriched			
% diff.	1.38	0.87	2.37
$P <$	0.025	N.S.	0.0125
Non-tested against tested			
% diff.	0.43	1.22	1.81
$P <$	N.S.	N.S.	N.S.

Two principal conclusions emerge. First, that many of the environmentally induced structural and behavioural changes induced in the developing animal can be maintained for a very much longer period than was previously thought probable, and second, that the rat's brain retains its capacity for structural change into old age. On the latter point it would seem that the long period of stimulus deprivation may have actually made the isolated animals more susceptible to stimulation since similar periods of behavioural testing have not been shown to induce changes of such magnitude in young animals³. Certainly the isolated animals showed a marked aversion to handling during the first few days of behavioural testing and generally seemed to be more aroused during each test period than their previously enriched littermates.

Evidence for structural plasticity was supported by changes in both brain weight and cerebral dimensions in response to sensory stimulation from either the rearing environment or the testing situation. It is of interest, however, that there did seem to be some ceiling effect imposed upon the environmentally-induced changes since the period of testing had no detectable effect on the previously enriched brains and only succeeded in increasing the size of the previously isolated brains to that of the enriched. Such a ceiling effect is consistent with the hypothesis that these neurological changes were the result of a genetic $\times$

environment interaction such that the extent of the neurological adaptation to a stimulus-rich environment was limited by a genetically imposed maximum; a maximum which had, presumably, been reached by the enrichment phase alone.

The resulting picture is, therefore, one of a brain dynamically reacting to an increased level of environmental stimulation by neurological change, indicating that the rat brain retains a considerable degree of structural plasticity into old age.

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R. A. CUMMINS
R. N. WALSH
O. E. BUDTZ-OLSEN
T. KONSTANTINOS
C. R. HORSFALL

Department of Physiology,
University of Queensland,
St Lucia,
Brisbane

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Acetylcholine Release within the Cat Striatum during the Sleep-Wakefulness Cycle

THE caudate nucleus (CN) is a subcortical region rich in acetylcholine (ACh)¹. Considering the role played by this nucleus² in sensorimotor control, changes in its cholinergic activity could occur during different stages of sleep (S) and wakefulness (W). Involvement of the CN in the S-W cycle is likely as electrical stimulation of the neostriatum results in S in various species³⁻⁵. We have therefore investigated the ACh output from caudate neurones during W, slow-wave sleep (SWS) and paradoxical sleep (PS) using chronic push-pull cannulae.

In five cats of either sex (2.5–3.0 kg), the following electrodes were implanted under pentobarbital anaesthesia (35 mg kg⁻¹ intraperitoneally): (1) Bipolar electrodes (0.4 mm diameter) in one lateral geniculate body (coordinates: $L=10$, $A=6$, $H=+2.5$ (ref. 6)); (2) bilateral cortical (occipital) screw electrodes; (3) bilateral EMG electrodes in the lateral rectus muscle of both eyes; (4) bilateral EMG loop electrodes in the neck muscles. A concentric push-pull cannula (internal diameter of the outer and inner cannula = 0.9 and 0.2 mm respectively) was inserted in the head of one CN ($A=16$, $L=3.8$, $H=+4.5$ (ref. 6)).

Three to six days later recording and perfusion sessions were performed with the animal in a ventilated, sound-attenuated chamber (60 × 60 × 60 cm). EEG and EMG were recorded by an eight-channel Grass polygraph. The CN was perfused through the push-pull cannula at 0.1 ml min⁻¹ with a warmed physiological Ringer-solution by means of a constant pressure perfusion pump (Ismatec M13). Individual 2 min samples were collected in a chilled fraction collector and ACh was determined radioenzymatically as described previously⁷. In some preliminary experiments neostigmine added to the perfusion fluid (5×10^{-4} M) did not result in changes in ACh output and so

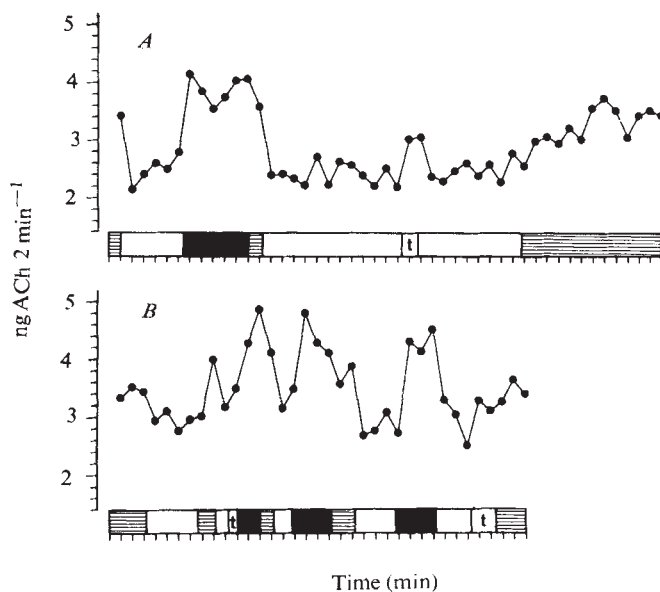


Fig. 1 Output of acetylcholine from the caudate nucleus as related to the sleep-wakefulness cycle during two different (A and B) perfusion sessions in one cat. The intervals on the abscissa indicate 2 min. (Transition = polygraph pattern intermediate between SWS and PS (A) or phase changes during a single sampling (B).) (□), wakefulness; (▤), slow-wave sleep; (■), paradoxical sleep; (t), transition.

was not further used. After one or two perfusion sessions each animal was killed by an overdose of pentobarbital and the localization of the cannula and electrodes verified histologically. Output of ACh was correlated with the concomitant changes in behaviour, EEG and EMG, characterizing W, SWS and PS according to the commonly accepted criteria^{8,9}.

The output of ACh from the CN and the phases of the S-W cycle during two typical perfusion experiments in one cat are shown in Fig. 1. Figure 1A depicts relatively prolonged, homogeneous phases of W, SWS and PS, whereas Fig. 1B illustrates shorter and more rapidly fluctuating phases. In all instances, ACh output decreased during SWS and increased during PS when compared to W. These changes occurred rapidly and in parallel to those of the electrical activities, and lasted as long as the various phases, irrespective of their duration. The statistical results are presented in Table 1.

It is evident that the differences in ACh output during W, SWS and PS were not only qualitatively the same in all cats,

Table 1 Output of ACh from the Caudate Nucleus of Cats during Wakefulness, Slow-wave Sleep and Paradoxical Sleep

Cat	Perfusion sessions	W ng/2 min	SWS* ng/2 min	PS* ng/2 min
1	A	3.12 ± 0.08 (32)†	2.10 ± 0.10 (19)	—
2	A	2.60 ± 0.12 (14)	2.24 ± 0.12 (12)	3.88 ± 0.12 (14)
	B	3.38 ± 0.08 (22)	2.32 ± 0.10 (12)	4.08 ± 0.12 (26)
3	A	3.80 ± 0.06 (5)	—	4.50 ± 0.06 (6)
	B	3.92 ± 0.14 (18)	3.20 (3.12, 3.28)	5.92 ± 0.18 (8)
4	A	3.34 ± 0.06 (12)	2.44 ± 0.08 (22)	3.90 ± 0.08 (6)
	B	3.58 ± 0.08 (9)	2.84 ± 0.06 (17)	4.58 ± 0.10 (8)
5	A	3.18 (3.22, 3.14)	2.54 ± 0.08 (8)	4.42 ± 0.06 (7)
Mean of the light perfusion sessions		3.38 ± 0.16	2.52 ± 0.16	4.46 ± 0.30

* All values during SWS and SP are significantly different from those during W ($P < 0.01$) either within each experiment (in which five or more samples were obtained) or as mean of the eight perfusions.

† Number of determinations indicated in parenthesis. For sessions in which less than five determinations were available individual values are given.

but also, and most importantly, the magnitude of these changes and the absolute ACh output during each phase were quantitatively similar. Thus, the output of ACh from the CN is greatly dependent upon the state of the W-SWS-PS cycle. The 2 min sampling period was too long, however, to measure accurately the biochemical changes during rapid transition between phases. In fact, when these periods occurred in the middle of a single sample collection, the corresponding ACh values were intermediate between those of the preceding and the following phases (Fig. 1B). This indicates further that the ACh output varies rapidly during changes in the W-SWS-PS cycle. Another type of transition occurred when a change from SWS to PS failed to develop fully, resulting in ACh release which was also intermediate between those of SWS and PS (Fig. 1A).

Previously, the ACh released from the cortex into a perfusion cup had been determined only in the presence of ACh-esterase inhibitors^{10,11}. In our experiments, these compounds did not influence the release of ACh from the CN, possibly due to a rate of wash-out of ACh from the tissue greater than its hydrolysis.

The involvement of ACh in the S-W cycle is indirectly suggested by previous reports. Thus, during SWS the release of ACh from the cerebral cortex decreases as compared with that during W and PS^{10,11}. Application of ACh crystals in brain regions, including the striatum¹², induced behavioural and electrographic manifestations of S. Moreover, the neostigmine-induced cortical desynchronization accompanying states of W was blocked by atropine^{11,13} which was also able to prevent the appearance of PS⁸. Microinjections of ACh into the brain stem reticular formation caused EEG synchronization and S¹⁴. The participation of the CN in the W-SWS-PS cycle has some basis from previous studies. This structure may counteract the action of the ascending reticular formation and thus intervene in the mechanism of W¹⁵. Both electrical stimulation³⁻⁵ of, and ACh application¹² into, the CN in different species induces a state of motor inactivity and eventually S.

This is the first study to examine the release of ACh from a subcortical structure (CN) during physiological changes in the chronically implanted, unrestrained, non-medicated cat. We have related the concepts of cholinergic and striatal involvement in the W-SWS-PS cycle and shown directly that the ACh output within the CN is smaller in SWS and greater in PS than in W. In addition, the rate of release of ACh during a phase of homogenous electrical activity (W, SWS or PS) is remarkably constant. These results may explain some clinical findings: thus, in extra-pyramidal disorders involving an imbalance between cholinergic and monoaminergic neurones (for example, Parkinson's syndrome)^{16,17} many symptoms remit during S^{18,19}. This may be the result of reduction of ACh release during SWS.

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M. GADEA-CIRIA*
H. STADLER
K. G. LLOYD
G. BARTHOLINI

Medical Research Department,
F. Hoffmann-La Roche and Co. Ltd,
4002 Basle

Received February 7; revised February 23, 1973.

* Present address: Departamento de Neurologia, Gran Hospital del Estado, Madrid, Spain.

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Development of Glucose Oxidation in Isolated Nerve Endings

A HIGH proportion of oxidative metabolism in the cerebral cortex is thought to occur in dendritic areas which are rich in synapses^{1,2}. With the development of methods to prepare isolated nerve endings (synaptosomes) from brain³, it has become possible to investigate the characteristics of oxidative metabolism at the synapse without significant glial contamination^{4,5}. Glucose is taken up into synaptosomes by a high-affinity carrier-mediated transport process⁶ and subsequently oxidized to support synthesis of ATP and phosphocreatine^{4,5}. Mitochondria probably do not metabolize glucose directly^{4,5} and glucose transport and metabolism are largely abolished if synaptosomes are ruptured by hypotonic shock^{4,6}.

Isolated nerve endings can be used to study the effect of added cation on transport and metabolism because, unlike brain slices, synaptosomes are relatively stable and metabolically active in buffered sucrose. We have learned that Na⁺ added to the incubation medium is not required for transport of 2-deoxy-D-glucose into synaptosomes⁶. By extrapolation from this observation, the findings reported here suggest that added Na⁺ stimulates glucose oxidation by 180% after glucose has entered nerve endings prepared from adult brain, and the primary effect is not on transport itself. Also, Na⁺ stimulation of glucose oxidation is minimal in synaptosomes prepared from immature brain but is acquired during maturation by respiring synaptosomes.

Synaptosomes were prepared from rat brain^{3,7} and inspected by electron microscopy by Dr J. R. Baringer of this department. They resembled synaptosomal fractions in published reports. Aliquots of synaptosomal suspension (0.4 ml) containing 2 mg protein were incubated in duplicate in 2.1 ml of a solution,

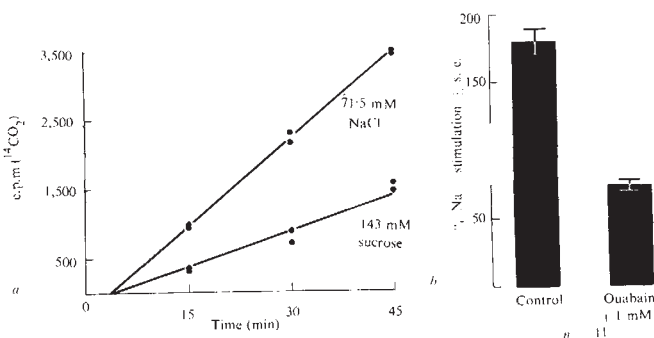


Fig. 1 a, Time course for glucose oxidation in rat brain synaptosomes in the presence or absence of Na⁺. Conditions are as described. b, Effect of ouabain on the percentage Na⁺ stimulation of glucose oxidation. The percentage is calculated by subtracting 1 from the quotient c.p.m. (71.5 mM Na⁺) ÷ c.p.m. (143 mM sucrose) and multiplying by 100.

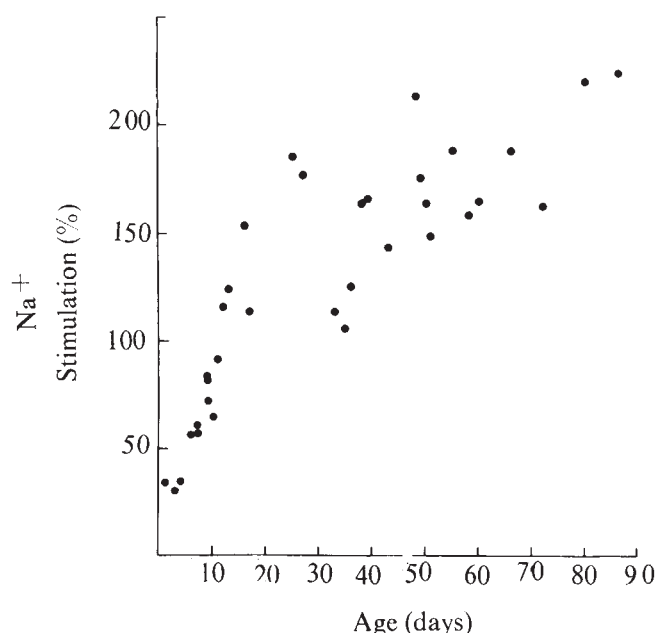


Fig. 2 Development of glucose oxidation in rat brain synaptosomes. The percentage Na^+ stimulation is calculated as in Fig. 1. Each point represents the average of duplicate measurements from a single animal or brain pooled from the same litter.

pH 7.4, containing 71.5 mM NaCl, 47.6 mM sucrose, 24.8 mM potassium phosphate buffer and 9.5 mM $\text{U-}^{14}\text{C}$ -glucose, 3.8×10^7 c.p.m. mmol^{-1} . Control flasks did not contain synaptosomes. Incubation was carried out in stoppered 25 ml Erlenmeyer flasks (Mettlglas Co., Boston) in a rotary water bath at 150 r.p.m. for 30 min at 37°C . Flasks had a centre well which contained a pleated 3×2 cm Whatman No. 1 filter paper saturated with 0.1 ml of 10% KOH to trap evolved $^{14}\text{CO}_2$. The reaction was stopped by injecting 0.5 ml of 0.5 M H_2SO_4 through the serum stopper into the incubation mix surrounding the centre well. After equilibration for 30 min filter papers were transferred directly to counting vials containing a 'Triton'-toluene scintillation solution for determination of radioactive $^{14}\text{CO}_2$ retained on the paper. Recovery of evolved $^{14}\text{CO}_2$ from ^{14}C -carbonate was nearly 100% and the assay was linear for protein concentration.

When synaptosomes from adult rats were incubated with ^{14}C -glucose, evolution of $^{14}\text{CO}_2$ was linear to 40 min whether or not NaCl (71.5 mM) was substituted for sucrose (143 mM) (Fig. 1a). Addition of MgCl_2 (1 mM) was without effect. Addition of NaCl resulted in a 180% stimulation of glucose oxidation (Fig. 1b). This effect appeared to be specific, for Na^+ as KCl and choline Cl at the same concentration were without effect.

Glucose oxidation may be increased in the presence of Na^+ to replenish ATP consumed by $\text{Na}^+\text{-K}^+\text{ATPase}$. As ouabain inhibits $\text{Na}^+\text{-K}^+\text{ATPase}$, ouabain should inhibit Na^+ -dependent glucose consumption in synaptosomes without affecting glucose oxidation in the absence of Na^+ . Ouabain (1 mM) did not alter glucose oxidation by synaptosomes in the absence of Na^+ in eleven separate experiments. In the presence of Na^+ , however, ouabain reduced Na^+ -stimulated glucose oxidation by 60% (Fig. 1b). These results suggest that Na^+ -dependent glucose oxidation in synaptosomes may be linked to membrane $\text{Na}^+\text{-K}^+\text{ATPase}$ and ADP production. 2,4-Dinitrophenol (0.1 mM) stimulates glucose oxidation in synaptosomes by 379% when regulation by ADP is removed. Thus, nerve endings probably have respiratory control, and fluctuations in Na^+ which attend changes in excitation and synaptic transmission may play an important role in regulating energy metabolism in nerve endings. Similar conclusions have been reported by Verity using glutamate⁵.

Nerve endings contain $\text{Na}^+\text{-K}^+\text{ATPase}$, and there is an

increase in synaptosomal $\text{Na}^+\text{-K}^+\text{ATPase}$ activity with maturation^{5,8}. We explored the development of Na^+ -dependent glucose consumption in nerve endings by preparing synaptosomes from the brain of developing rats. There was a rapid increase in Na^+ -dependent glucose oxidation with development, but glucose oxidation in the absence of Na^+ did not change as dramatically with age. These results are expressed as the percentage of glucose oxidation stimulated by Na^+ (71.5 mM) compared with sucrose (143 mM) (Fig. 2). The marked increase in this percentage with maturation cannot be explained by recovery of greater numbers of synaptosomes with age. Development of Na^+ -dependent glucose oxidation correlates roughly with the appearance of $\text{Na}^+\text{-K}^+\text{ATPase}$ in the synaptosomal fraction⁸. Several possibilities may account for the development of Na^+ -dependent glucose oxidation in isolated nerve endings. During maturation different populations of synaptosomes might be recovered from the brain at different stages of development, there may be activation of a latent form of $\text{Na}^+\text{-K}^+\text{ATPase}$ in nerve endings, or during development newly synthesized $\text{Na}^+\text{-K}^+\text{ATPase}$ may be inserted separately into existing synaptosomal membranes.

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I. DIAMOND

Departments of Neurology and Pediatrics,
University of California
School of Medicine,
San Francisco, California 94122

R. A. FISHMAN

Department of Neurology,
University of California
School of Medicine,
San Francisco, California 94122

Received December 27, 1972; revised February 26, 1973.

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Effect of Lithium on Brain Dopamine

THE efficacy of lithium ion in the therapy of mania is now established^{1,2}, and much speculation about its mode of action has involved its effects on brain catecholamine metabolism. Treatment with lithium ion increases the turnover rate of whole brain noradrenaline³⁻⁴; increases intraneuronal while decreasing extraneuronal metabolism of noradrenaline^{3,4}; increases uptake of noradrenaline by synaptosomes⁵, and reduces the rate of release of ^3H -noradrenaline caused by electrical stimulation of striatal slices⁶. The influence of lithium ion on brain dopamine, however, is less fully documented. Here we describe the effect of chronic lithium chloride treatment on dopamine synthesis in striatal brain slices.

Male albino Sprague-Dawley rats (180 to 200 g) were housed four per cage in a regime of 12 h light and 12 h darkness and fed lab chow and water *ad lib*. Lithium chloride, dissolved in distilled water (0.7% w/v) or an equal volume of isotonic sodium chloride, was administered intraperitoneally (i.p.) as acute or chronic daily injections. Animals were killed 60 min after the last injection, the brains were quickly removed, rinsed in ice-cold Krebs-Henseleit physiological solution and the

striatum was dissected out as described by Glowinski and Iversen⁷. Striatal slices (0.4 mm thick) were prepared using the slicing guide described by McIlwain⁸ and placed in flasks containing 2 ml. of oxygenated cold physiological solution. Incubations were carried out at 37° C in an atmosphere of 95% O₂-5% CO₂. After a 10 min preincubation, 3,5-³H-tyrosine (New England Nuclear Corp.) was added to each flask to give a final concentration of 8.15×10^{-6} M tyrosine and the incubations were continued for a further 45 min. The tissue and incubation media were rapidly separated by filtration and the tissue slices were washed twice with cold physiological solution. The tissue was homogenized in 0.4 N perchloric acid and the media and washings were acidified to give a final 0.4 normality with perchloric acid. The samples were centrifuged and cold tyrosine and dopamine were added to the supernatant as carriers. Labelled and endogenous tyrosine and dopamine were isolated on 'Dowex' 50-4X columns (K⁺ form) followed by purification with alumina as described by Neff *et al.*⁹. Aliquots of the tyrosine and dopamine were taken for radioactive determination by liquid scintillation spectroscopy. Endogenous amines were determined fluorometrically by methods described previously^{10,11}. Fluorescence was read in the Aminco Bowman spectrofluorometer. Plasma lithium concentrations were determined by atomic absorption spectrometry.

Table 1 Effect of LiCl on Dopamine Synthesis in Striatal Slices

Treatment	N*	Dose (mequiv kg ⁻¹)	Plasma Li ⁺ (mequiv l. ⁻¹)	Dopa-mine (μg g ⁻¹)	³ H-Dopa-mine (c.p.m. mg ⁻¹)
Acute NaCl	16	—	—	4.21 ± 0.08	1,063 ± 21
LiCl	10	2	1.65	4.34 ± 0.05	1,108 ± 28
LiCl	10	4	2.84	4.28 ± 0.06	1,162 ± 31
Chronic NaCl	8	—	—	4.02 ± 0.07	1,160 ± 44
LiCl	8	1	0.64	3.89 ± 0.07	761 ± 25†
LiCl	8	2	1.52	3.82 ± 0.05	445 ± 24‡

* Number of animals; each provided duplicate striatal samples.

† $P < 0.005$.

‡ $P < 0.001$.

Acute injections of lithium chloride in doses of 2 to 4 mequiv kg⁻¹ did not affect endogenous dopamine nor the synthesis of ³H-dopamine in striatal slices (Table 1). Daily administration of lithium chloride, 1 and 2 mequiv kg⁻¹ for 14 days, inhibited striatal dopamine synthesis by 34 and 62% respectively (Table 1). These doses caused a slight but insignificant decrease in endogenous dopamine. There was no alteration in tyrosine specific activity. Serum lithium concentrations of 0.54 to 1.7 mequiv l.⁻¹ resulted from the chronic treatment; these levels are within the range associated with lithium therapy. The *in vitro* addition of 4 to 18 mequiv l.⁻¹ of lithium ion, or the replacement of similar amounts of sodium by lithium in the media incubating striatal slices obtained from saline-treated rats, did not affect dopamine synthesis (Table 2).

Table 2 Effect of *in vitro* LiCl or Replacement of Na by Li Ion on Striatal Dopamine Synthesis

Concentration of Li ⁺ (mequiv l. ⁻¹)	N*	³ H-Dopamine (c.p.m. mg ⁻¹)
Added to control media	6	948 ± 24
4	4	1,028 ± 34
6	4	1,006 ± 29
Replacement of Na	6	
4	4	959 ± 28
6	4	1,078 ± 31
18	4	992 ± 32

* Number of animals; each provided duplicate striatal samples.

Thus the inhibition of dopamine synthesis by lithium ion seems to require chronic treatment. High lithium concentrations produced by either an acute injection or by *in vitro* addition of lithium ion are insufficient to inhibit synthesis. These con-

clusions correlate with the clinical observations that lithium's anti-manic action requires 1 to 2 weeks of daily medication. Our results agree with the observed decrease of dopamine excretion in manic patients undergoing lithium treatment¹². Although the clinical results may reflect the effect of lithium on the peripheral disposition of dopamine, the data reported here suggest that central dopamine metabolism is altered in the same direction by lithium treatment.

Corrodi *et al.*¹⁴ also found a slight but significant decrease in whole brain dopamine turnover in rats treated with lithium for 3 weeks, judged on the basis of the rate of dopamine decline observed after injection of α -methyl-*p*-tyrosine. They have further found, using the histochemical fluorescence method, an increase in tubero-infundibular dopamine turnover. On the other hand, Ho *et al.*¹⁶ found no alteration in dopamine turnover rates in four brain regions after 4 weeks of treatment with lithium. They used the synthesis inhibition method for estimating dopamine turnover. Although differences in methods and treatment time may have contributed to the discrepancy in these results, the fact that different brain regions were studied may be more significant, especially as we have been concerned only with the corpus striatum.

Messiha *et al.* reported increased urinary levels of dopamine during manic states¹², while others have described the induction of hypomanic and manic behaviour by L-dopa in depressed patients with prior episodes of mania¹³. Thus, the inhibition of striatal dopamine synthesis may play some part in the psychomotor action of lithium in mania, or this element may exert some more fundamental action on the disorder itself.

EITAN FRIEDMAN

SAMUEL GERSHON

*Neuropsychopharmacology Research Unit,
New York University Medical Center,
550 First Avenue,
New York, New York 10016*

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L-Glutamic Acid Decarboxylase in Parkinson's Disease: Effect of L-Dopa Therapy

In Parkinson's disease severe decreases occur in striatal L-dopa decarboxylase, putaminal synaptosomal uptake of dopamine, and dopamine and homovanillic acid concentrations in the nigro-striato-pallidal complex¹⁻⁴. In addition, L-glutamic decarboxylase (GAD) activity is decreased in the caudate nucleus⁵. The experiments described here were performed to further evaluate this finding and to determine the effect (if any) of L-dopa therapy.

Table 1 Activity of L-Glutamic Acid Decarboxylase in Discrete Brain Regions of Control Patients and Patients with Parkinson's Disease

Caudate nucleus	Putamen	Pallidum externum	Substantia nigra	Temporal cortex
<i>A, Control patients</i>				
1,318 ± 185 (13)	1,243 ± 220 (13)	1,106 ± 306 (13)	1,273 ± 297 (13)	1,024 ± 154 (13)
<i>B, Patients with Parkinson's disease</i>				
<i>1. No L-dopa therapy</i>				
641 (2)	583 (2)	776 (2)	526 (2)	663 (2)
<i>2. L-Dopa therapy for 8 months or less (average dose: 3 g day⁻¹)</i>				
339 ± 53 (4) *	249 ± 24 (4) *	504 ± 50 (4)	300 ± 102 (4) †	292 ± 101 (3) *
<i>3. L-Dopa therapy for 1 yr or longer (average dose: 4 g day⁻¹)</i>				
1,172 ± 173 (5) ‡	887 ± 95 (5) §	1,210 ± 109 (5) §	1,210 ± 110 (4) §	407 ± 67 *

Patients on L-dopa received the drug for different periods in daily oral doses ranging from 2 to 6 g. Enzyme activity (mean ± s.e.m.) is expressed as nmol carbon dioxide produced per 100 mg of protein per 2 h. (Number of cases in parentheses.) Brain tissue was homogenized in ice-cold isotonic dextrose and incubated for 20 min (37° C) in the presence of phosphate buffer (pH 7.0) and 0.6 mM pyridoxal phosphate. L-Glutamic acid was then added (final concentration of 2.5 mM containing 0.4 µCi DL-glutamic acid-1⁴COOH) and after 2 h the reaction was terminated by addition of acid. The evolved carbon dioxide was trapped in hyamine hydroxide and then assayed for radioactivity.

* Significantly different from group A ($P < 0.01$).

† Significantly different from group A ($P < 0.001$).

‡ Significantly different from group B, 2 ($P < 0.05$).

§ Significantly different from group B, 2 ($P < 0.001$).

Human or rat brains were dissected and stored on dry ice as previously described⁶. Human material was obtained at autopsy from either control (no known neurological disorder) or Parkinsonian patients. Nine Parkinsonian patients received L-dopa until they died; two others did not receive L-dopa therapy. The patient groups were well matched with respect to age (means: control=68 yr; Parkinsonian=67 yr), sex (controls: ten males and three females; Parkinsonian: nine males and two females) and interval between death and freezing of the tissue (4–21 h for the controls, and 4–17 h for the Parkinsonian patients). None of these parameters had any apparent influence on GAD activity⁴.

For the animal experiments, male albino Wistar rats (250–275 g) were fed an aqueous suspension of L-dopa by way of a stomach tube on the following regimen: days 1–37, 50 mg per rat per day; days 38–88, 100 mg per rat per day; days 89–109, 2 × 100 mg per rat per day. Controls were fed the suspension fluid (water) according to the same schedule. Animals were killed at days 37, 88 and 109. The striata were immediately removed and frozen.

GAD activity was estimated as described for L-dopa decarboxylase⁶. In brief, brain tissue was homogenized in ice-cold isotonic dextrose and incubated at pH 7.0 (37° C, 60 min) in the presence of pyridoxal phosphate (0.6 mM) and L-glutamic acid (2.5 mM containing 0.4 µCi DL-glutamic acid-1⁴C). The carbon dioxide formed was trapped in hyamine hydroxide and assayed for radioactivity. Blanks contained *p*-bromo-*m*-hydroxybenzylamine, a potent inactivator of pyridoxal phosphate⁷.

The GAD activity in the striatum and substantia nigra of the non-dopa treated Parkinsonian patients was less than 50% of the control mean (Table 1), in agreement with a previous report¹. In other brain regions GAD activity was within the range of the control values. In the brains of patients who received L-dopa for 8 months or less, GAD activity was significantly lower than in the controls (Table 1). In the caudate nucleus, putamen, substantia nigra, and globus pallidus of patients who received L-dopa continuously for 1 yr or longer, however, GAD activity was within the range of the controls, being significantly greater than that of patients treated for a shorter time. The GAD activity in other brain regions was only slightly higher in patients with the more prolonged L-dopa treatment.

In the rats treated chronically with oral L-dopa, after administration of L-dopa for 37 and 38 days the striatal GAD activity was not different from control (Table 2). After 109 days, however, striatal GAD activity was significantly

greater than in either those rats which had received lower doses of L-dopa for a shorter period, or the controls. Thus, it was possible to replicate, in controlled laboratory conditions, the increase in striatal GAD activity observed in Parkinsonian patients chronically treated with L-dopa.

The GAD activity of the substantia nigra and globus pallidus, but not striatum, in Parkinsonism was reported to be subnormal compared with the frontal cortex^{8,9}. Absolute GAD activity was, however, decreased throughout the brain compared with controls. Moreover, there was no indication of whether or not L-dopa had been administered. We find that in Parkinson's disease there is a definite decrease in the activity of striatal GAD and that its amelioration by L-dopa therapy is dependent upon the daily dose of L-dopa and the duration of its administration. This implies that the striatal GAD-L-dopa relationship depends on the concentration of dopamine present. In fact, in rats the increase in striatal GAD induced by L-dopa was dose-dependent. In addition, the L-dopa-treated patients with highest GAD activities were treated for longer (1 year or longer) and received, as a group, a higher dose of L-dopa (Table 1). It has also been shown that the striatal dopamine concentration of chronically L-dopa treated patients is markedly higher than in non-dopa treated patients^{5,12,13}. Correspondingly, the more chronically treated group of Parkinsonian patients had an average concentration of striatal dopamine (caudate nucleus: 1.6 µg g⁻¹) distinctly higher than in the

Table 2 Effect of Chronic Oral L-Dopa Administration on the GAD Activity of the Rat Striatum

Duration of L-dopa administration (days)	L-Glutamic acid decarboxylase activity		
	Mean ± s.e.m.	No. of animals	% of control
Control	7,399 ± 950	7	100.0
37 days	7,360 ± 853	7	99.5
88 days	7,012	2	94.8
109 days	11,310 ± 839	5	152.8 * †

Rats were fed an L-dopa suspension (on the regimen described in detail in the text) by means of a metal stomach tube at 1600 h (days 1–88) or at 0800 and 2000 h (days 89–109). Enzymic activity expressed as nmol carbon dioxide produced per 100 mg of protein per 2 h. Age-matched sham-treated control animals were killed with each experimental group and GAD values did not differ between groups; hence all control values were combined. Assay conditions as in Table 1. * Significantly different from controls, $P < 0.002$. † Significantly different from 37+88 day groups, $P < 0.005$.

group treated for 8 months or less (caudate nucleus: $0.7 \mu\text{g g}^{-1}$).

What is the mechanism of the L-dopa-induced increase in GAD activity? Although large diencephalic lesions in experimental animals decrease GAD activity in the substantia nigra and globus pallidus^{8,10}, no such lesions are apparent in Parkinson's disease¹¹. Thus the decrease in GAD activity in Parkinson's disease may be secondary to the severe degeneration of the nigrostriatal dopamine pathway. These GAD-containing neurones could be under a continuous "trophic" influence of the dopaminergic system; degeneration of the dopaminergic pathway might then result in a biochemical "atrophy" of the GAD neurones. Replenishment of striatal dopamine by L-dopa^{4,14} possibly reverses the biochemical atrophy of the GAD-containing neurones, a process that may well require prolonged administration of L-dopa.

Dopamine might act by removing a repression of GAD synthesis. This is an attractive but possibly incomplete explanation because of the long latency before striatal GAD activity increases. Relatively high concentrations of striatal dopamine may be needed, a process requiring a considerable time. This is supported by the lengthy period of L-dopa administration required to increase striatal GAD activity in rat brain.

An alternative hypothesis is an enhanced synthesis of GAD apoenzyme in compensation for decreased cofactor availability as a result of Schiff base formation with L-dopa^{15,16}. Such an *in vivo* formation of Schiff base would not be reflected *in vitro* where an optimal concentration of pyridoxal phosphate is added. Signs of vitamin B₆ deficiency are not, however, apparent during chronic L-dopa therapy, making this a rather unlikely hypothesis.

The increase in striatal GAD activity during chronic L-dopa therapy may have an important clinical correlate as it conspicuously parallels the ameliorative effect of L-dopa on Parkinsonian tremor (which may take from several weeks to months to develop¹⁷). So far the anti-tremor effect of L-dopa has been without any known neurochemical correlate; our observations suggest that a GABA-containing neurone system may be involved. In this context, a new GABA-like compound has recently been reported to exert a specific anti-tremor effect in Parkinsonian patients¹⁸.

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K. G. LLOYD*
O. HORNYKIEWICZ

Department of Psychopharmacology,
Clarke Institute of Psychiatry,
and Department of Pharmacology,
University of Toronto,
Toronto,
Ontario

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* Present address: Department of Medical Research, F. Hoffmann-LaRoche and Co. Ltd, 4002 Basle, Switzerland.

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pH-Dependent Changes in Composition of Carcinoembryonic Antigen

CARCINOEMBRYONIC antigens (CEA) are present in perchloric acid-soluble extracts of carcinoma of the gastrointestinal tract, particularly of the colon and rectum¹. I report here some observations suggesting that CEA, as present in perchloric acid extracts of carcinomata, is an aggregate of cationic protein and metachromatic material.

Tissue was homogenized in five to ten volumes of saline, an equal volume of 1.2 M perchloric acid added, the mixture centrifuged and the supernatant neutralized with 1 N NaOH; all procedures were carried out at 4° C and with vigorous stirring. Extracts were concentrated to minimal volumes in an ultrafiltration cell using a UM-10 'Diaflo' filter (Amicon Corporation, Lexington, Massachusetts). Three rabbits were given thirteen weekly injections of pooled extracts from nine carcinomata of the colon or rectum, initially combined with Freund's complete adjuvant and then with incomplete adjuvant. Thirty millilitres of pooled antiserum was absorbed with perchloric acid extracts of normal colon and rectum, a mucoprotein fraction from pooled human serum and fractions of human serum. The large and small intestine were obtained within 12 h of death from two subjects (one dying from a gunshot wound, the other from a coronary occlusion), the mucosa and the remainder of the bowel wall were extracted separately and the extracts concentrated to 7.6 ml and 4.2 ml respectively. Mucosa extract (5.1 ml) and 2.3 ml of the muscle coat extract were added to the 30 ml of antiserum. Two serum mucoprotein fractions were prepared from 1,250 ml and 670 ml of human serum by adding an equal volume of 0.4 M perchloric acid, centrifuging and neutralizing the supernatant with 0.5 N NaOH; each was concentrated to approximately 10 ml, and 2.6 ml and 3.4 ml respectively were added to the antiserum. Seven fractions of human serum obtained by gel and ion exchange chromatography were added in amounts ranging from 0.1–17.6 mg of protein ml⁻¹ of antiserum.

By Ouchterlony double diffusion, the absorbed antiserum revealed two antigens in extracts of eighteen surgical specimens of carcinomata of the colon or rectum (Fig. 1A). One of these antigens was identified as CEA by an antiserum obtained from Dr P. Gold, Montreal General Hospital (Fig. 1A, B). The second antigen has antigenic sites in common with CEA (Fig. 1C) and will be called Ca-2. Extracts of macroscopically normal intestine from fourteen of the surgical specimens gave precipitin lines that merged with the CEA and Ca-2 lines given by the corresponding tumour extract. The mucosa extract from the two non-cancerous subjects also gave two very faint lines which merged with the two lines given by an extract of rectal carcinoma. When a portion of the antiserum was further absorbed with an extract of a rectal carcinoma it no longer reacted with any of the above extracts. Other workers have detected CEA in perchloric acid extracts of non-cancerous colon².

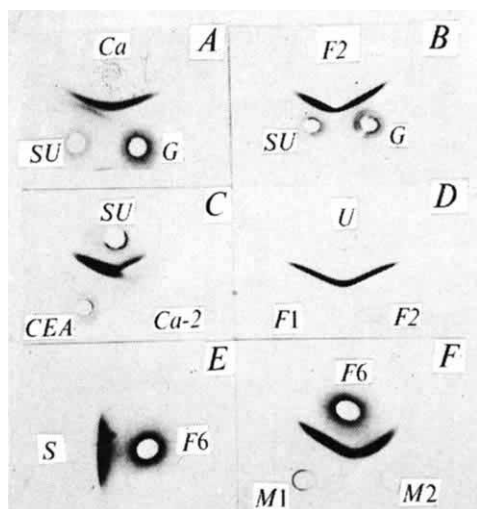


Fig. 1 Ouchterlony double diffusion reactions in 1% agarose. For *A*, barbital buffer¹⁶ was used. For the other plates, 2% agarose was mixed with an equal volume of the Tris-HCl buffer (see text). *SU*, Absorbed antiserum to colonic carcinoma; *G*, anti-CEA (goat 81); *U*, unabsorbed antiserum to colonic carcinoma. *A*: *Ca*, Concentrated extract of a colonic carcinoma. The *Ca-2* antigen (see text and *C* below) is indicated by the faint line between the CEA precipitin line and antibody well. *B*: *F2*, fraction 2, Fig. 3*B*. *C*: *CEA*, CEA preparation shown in Fig. 5*A*; *Ca-2*, the *Ca-2* antigen isolated from the same twenty-four carcinomata as CEA and by the same procedures. On gel chromatography *Ca-2* was eluted after CEA but was eluted first during chromatography on DEAE 'Sephadex'. During chromatography on SE 'Sephadex' and DEAE 'Sephadex' at pH 2.2, *Ca-2* partly dissociated into a cationic protein and metachromatic material as described for CEA in Fig. 5*B* and *D*. Note the spur given by the CEA precipitin line. *D*: *F1* and *F2*, fractions 1 and 2, Fig. 5*D*. An identical reaction was given by these two fractions and the absorbed antiserum. *E*: *F6*, fraction 6, Fig. 3*C*; *M1* and *M2*, two fractions isolated from the same extract as CEA and which contain metachromatic material. *M1* also contained a small amount of material staining with coomassie blue, but *M2* contained metachromatic material only. These spurious precipitin lines persisted even after washing the slides in 0.15 M NaCl for 4 d.

Pooled extracts from twenty-four carcinomata were chromatographed on a 60×490 mm column of 'Sephadex' G100 (Pharmacia, Uppsala) using a 0.05 M Tris-HCl buffer, pH 7.2, containing 0.5 M NaCl. Fractions were concentrated by ultrafiltration with a UM-10 'Diaflo' filter and chromatographed on a 35×120 mm column of DEAE 'Sephadex' equilibrated with 0.0075 M phosphate, pH 7.2. Proteins were eluted with a continuous NaCl gradient established by 0.01 M phosphate buffer, pH 7.2, containing 0.2 M NaCl. Fractions containing CEA were pooled and chromatographed on 'Sephadex' G200 (Fig. 2*A*) and then on 'Sephadex' 6B (Fig. 2*B*). The presence of more than one CEA peak after chromatography on 'Sephadex' G200 has been reported by other workers^{3,4}. 'Sephadex' 4B has also been used to isolate CEA and is eluted from it with a percentage column volume of 70% to 80%^{3,4}.

During the isolation, concentrated fractions were tested by (a) Ouchterlony double diffusion and immunoelectrophoresis using both the absorbed and unabsorbed antisera and (b) electrophoresis followed by staining with coomassie blue for protein, with toluidine blue for metachromatic substances⁵, or by staining for the periodic acid Schiff (PAS) reaction for carbohydrate⁵. CEA eluted from the 'Sephadex' 6B column contained no material staining metachromatically but contained a trace amount of a PAS positive substance migrating to the cathode (Fig. 3*A*). Antisera (Behringwerke AG) to six serum proteins soluble in perchloric acid (namely, α_1 -antitrypsin, α_2 -HS-glycoprotein, Zn- α -glycoprotein, β_2 -glycoprotein, haptoglobin and haemopexin) did not react with this CEA preparation in Ouchterlony double diffusion.

Immunoelectrophoresis revealed diffuse material and one precipitin arc merging with the CEA arc (Fig. 3*A*). Preparative electrophoresis in agarose failed to remove this additional material (Fig. 3*B*).

Fraction 2 (Fig. 3*B*), obtained by preparative electrophoresis, was chromatographed on DEAE 'Sephadex' at pH 5.7—that is, at a lower pH than that used during isolation of CEA from the cancer extracts. On immunoelectrophoresis the shape and position of the CEA arcs varied between different fractions eluted and from that of the starting material (Fig. 3*C*). Two fractions contained a cationic protein which had no specificity for CEA and which stained much more intensely with coomassie blue than CEA (Fig. 3*C*). Dissociation of CEA by a comparatively minor change in pH (from 7.2 to 5.7) suggests that the peculiar immunoelectrophoretic pattern (Fig. 3*A*, *B*) resulted from dissociation caused by the voltage applied during electrophoresis⁶.

When the cationic protein (fraction 6, Fig. 3*C*) was allowed to diffuse against other proteins in a manner analogous to Ouchterlony double diffusion, precipitates resembling immune precipitin lines were formed even in the presence of 0.25 M NaCl (Fig. 1*E*, *F*). When the three fractions retaining CEA specificity (fractions 2, 3 and 4, Fig. 3*C*) were pooled and tested in the same manner as above, this pooled material also gave a spurious precipitin line when allowed to diffuse against the metachromatic material but did not form a precipitate with the serum mucoprotein fraction.

Fraction 2 (*F2*, Fig. 2*A*), eluted after the main CEA peak on 'Sephadex' G200 chromatography, and which contained both CEA and the *Ca-2* antigen, was equilibrated with 0.01 M glycine-HCl buffer, pH 2.2, in an ultrafiltration cell with a UM-2 'Diaflo' filter. Filtrate from this cell gave a precipitate with 8% phosphotungstic acid solution. The precipitable material in this filtrate was restrained by a UM-05 'Diaflo' filter. When material retained by the UM-2 filter (see above)

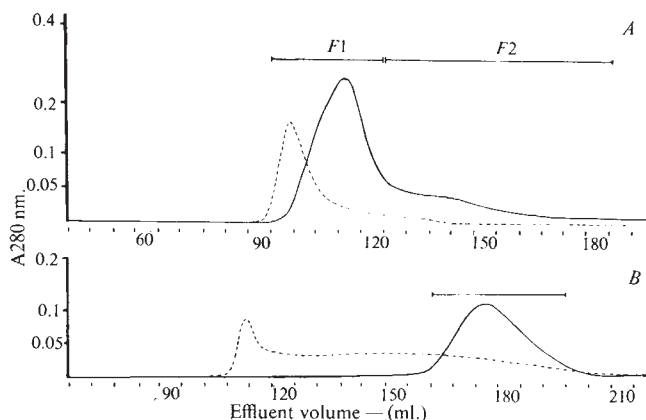


Fig. 2 Gel chromatography of CEA fractions. A 0.05 M Tris-HCl buffer, pH 7.2, containing 0.5 M NaCl, 0.02% sodium azide and 1% butanol was used for elution in each case. Eluate was monitored at 280 nm. Dimensions of the packed columns were 25×531 mm for the 'Sephadex' G200 and 25×541 mm for the 'Sephadex' 6B column. Six millilitre fractions were collected at a flow rate of 14.4 ml h⁻¹. Void volumes were obtained by chromatography of 'Blue Dextran' 2000 (Pharmacia, Uppsala). —, Recording given by CEA; ---, recording given by 'Blue Dextran'. *A*, Chromatography on 'Sephadex' G200 of the CEA preparation described in the text. The partition coefficient and % column volume for the main CEA peak (*F1*) were 0.08 and 35% respectively. Fractions under the line marked *F1* were concentrated with a UM-10 'Diaflo' filter and chromatographed on 'Sephadex' 6B (see *B*). The CEA in *F1* stained blue with toluidine blue but showed no metachromasia. Fractions under the line marked *F2* were pooled and concentrated with a UM-10 filter (see Fig. 4*A*). *B*, Chromatography on 'Sephadex' 6B of fraction *F1* in *A* above. CEA was eluted with a partition coefficient of 0.38 or a percentage column volume of 62%. Fractions under the horizontal line were pooled and concentrated with a UM-10 filter (see Fig. 3*A*).

was chromatographed on DEAE 'Sephadex' at pH 2.2, the greater part of the starting material was converted to proteins migrating to the cathode, some of which retained specificity for CEA but with weaker PAS reactions than the starting material (Fig. 4A, B).

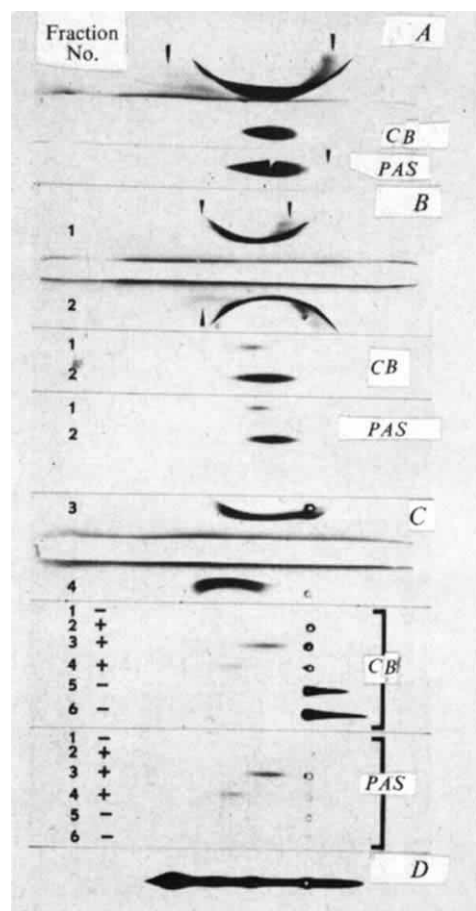


Fig. 3 Immunoelectrophoretic and electrophoretic patterns of concentrated fractions of CEA. Electrophoresis was carried out on glass slides (25×76 mm), using 1% agarose (General Biochemicals, Ohio) and a discontinuous barbital buffer, pH 8.6 (ref. 16), at a constant current of 5 mA per slide for 25 min. Unabsorbed antiserum to colonic carcinoma was used for all the immunoelectrophoretic patterns. Immunodiffusion was carried out for 48 h at 37°C . For electrophoretic patterns, the slides were fixed in methanol, dried and stained with coomassie blue (CB) or by the periodic acid Schiff (PAS) reaction for carbohydrate. Slides were stained for 15 min in 0.2% coomassie blue in methanol-water-acetic acid (50-40-10), and washed in methanol-water-acetic acid (50-145-5). Arrows indicate unusual features of the patterns and the contaminant detected by the PAS reaction. The detection of CEA by Ouchterlony double diffusion is recorded as positive (+) or negative (-). For all slides illustrated, the anode was on the left side. **A**, The concentrated CEA fraction obtained by chromatography on 'Sephadex' 6B (Fig. 2B). **B**, Two of three fractions obtained from the CEA fraction shown in **A** above by preparative electrophoresis in agarose. Electrophoresis was carried out as above, the agarose cut across to separate the leading, intermediate and trailing segments of the CEA region, and the separated portions eluted with 0.05 M Tris-HCl buffer, pH 7.2, containing 0.5 M NaCl. **C**, Fractions obtained by ion exchange chromatography of fraction 2 in **B** above. A column (25×340 mm) of DEAE 'Sephadex' was equilibrated with 0.005 M phosphate buffer, pH 5.7, as starting buffer. Fractions 1-4 were eluted by a continuous NaCl gradient. After gradient elution, the column was washed with (a) 1.0 M NaCl in phosphate buffer (fraction 5), and (b) 1.0 M NaCl in 0.01 N HCl (fraction 6). Immunoelectrophoresis with absorbed antiserum to colonic carcinoma gave the same patterns as those shown here for fractions 3 and 4 with the unabsorbed antiserum. **D**, Electrophoretic pattern of serum stained with coomassie blue.

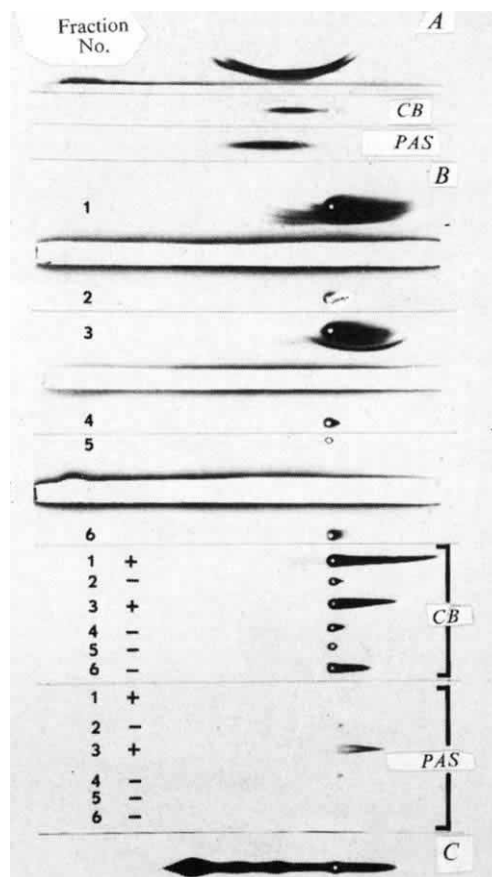


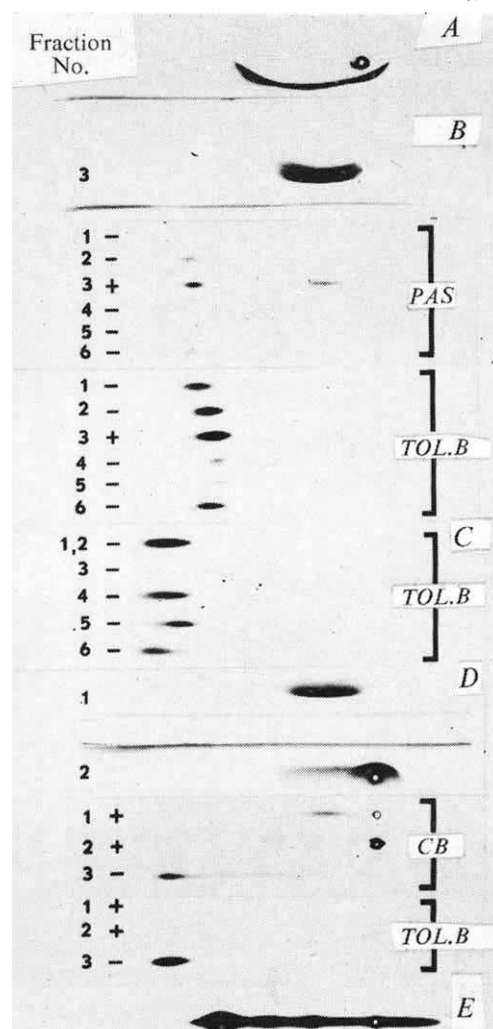
Fig. 4 Immunoelectrophoretic and electrophoretic patterns of concentrated fractions which contain both CEA and the Ca-2 antigen. Electrophoresis and immunoelectrophoresis were carried out as described in Fig. 3. CB, Coomassie blue stain; PAS, periodic acid Schiff stain for carbohydrate. The detection of Ca-2 and CEA by Ouchterlony double diffusion is recorded as positive (+) or negative (-). **A**, A fraction isolated by chromatography on 'Sephadex' G200 (F2, Fig. 2A). The precipitin arc due to the Ca-2 antigen is nearest the antibody trough, and the CEA arc lies above and parallel to it. **B**, Fractions isolated from F2 shown in **A** above by ion exchange chromatography on a column (25×355) of DEAE 'Sephadex' which was equilibrated with 0.01 M glycine-HCl buffer, pH 2.2. Material in fraction 1 passed through the column without being bound to the DEAE 'Sephadex'. Fractions 2-5 were eluted with a continuous NaCl gradient produced by 0.5 M NaCl in the same glycine buffer. Fraction 6 was then eluted from the column with 1 M NaCl in 0.01 M HCl. Note that for fraction 1, and to a lesser extent for fraction 3, continuous arcs run from the slow α_2 region through to the γ region of the immunoelectrophoretic patterns. Note also the loss of PAS reactivity in fractions 1 and 3 compared to the starting material. **C**, Electrophoretic pattern of serum stained with coomassie blue.

Fractions 2, 3 and 4, Fig. 3C, were pooled and chromatographed on a cation exchanger (SE 'Sephadex') at pH 2.2. The immunoelectrophoretic arc of the eluted CEA differed from that of the starting material while all the fractions eluted contained negatively charged, metachromatic material (Fig. 5A-C).

Finally, fraction 3 (Fig. 5B) from the chromatography on SE 'Sephadex' was chromatographed on DEAE 'Sephadex' at pH 2.2 (Fig. 5D). Immunoelectrophoresis revealed changes in the shape and position of the CEA arc from that of the starting material (fraction 2, Fig. 5D). A fraction eluted after CEA contained metachromatic material (fraction 3, Fig. 5D).

It is not clear whether any CEA determinants are covalently linked to the cationic protein. A basic protein that has been isolated from bovine spinal cord by extraction with HCl at pH 1.7⁷ acts as a natural receptor for N-acetyl-galactos-

Fig. 5 Immuno-electrophoretic and electrophoretic patterns of concentrated fractions of CEA carried out as described in Fig. 3. The immuno-electrophoretic patterns shown were produced by the unabsorbed antiserum to colonic carcinoma; identical patterns were given by the absorbed antiserum. The detection of CEA by Ouchterlony double diffusion is recorded as positive (+) or negative (-). PAS, Periodic acid Schiff reaction for carbohydrate; TOL B, toluidine blue stain for metachromatic substances⁵; CB, coomassie blue stain. *A*, Pooled CEA fractions (fractions 2, 3 and 4, Fig. 3C). *B*, Fractions obtained by ion exchange chromatography on SE 'Sephadex' of the CEA shown in *A* above. A column (25 × 280 mm) of SE 'Sephadex' was equilibrated with 0.025 M glycine-HCl buffer, pH 2.2, as starting buffer. Fraction 1 passed through without being bound. Fractions 2-5 were eluted by a continuous NaCl gradient produced by 1.0 M NaCl in the same glycine buffer. Fraction 6 was then eluted with 0.1 M glycine-NaOH buffer, pH 9.6, containing 1.0 M NaCl. Fractions were concentrated with a UM-10 'Diaflo' filter. The rapidly migrating material present in all six fractions was strongly metachromatic and faintly PAS positive. *C*, Material present in the UM-10 filtrates of the six fractions shown in *B* above and which was retained by a UM-05 filter. The UM-10 filtrates from fractions 1 and 2 in *B* above were pooled, while the remaining four UM-10 filtrates were concentrated with the UM-05 filter individually. The retained material was strongly metachromatic and faintly PAS positive. *D*, Fractions obtained by chromatography on DEAE 'Sephadex' of fraction 3 in *B* above. A column (15 × 200 mm) of DEAE 'Sephadex' was equilibrated with 0.025 M glycine-HCl buffer, pH 2.2. Fraction 1 was eluted in the starting buffer and fraction 2 by a continuous NaCl gradient using 0.6 M NaCl in the same glycine buffer. Fraction 3 was then eluted with 1 M NaCl in 0.01 N HCl. Fractions were concentrated with a UM-10 'Diaflo' filter. No difference could be detected between the CEA in fractions 1 and 2 by Ouchterlony double diffusion (Fig. 1D). The CEA in fractions 1 and 2 and the metachromatic material in fraction 3 were faintly PAS positive. When the UM-10 filtrates from the three fractions were concentrated separately with a UM-05 filter, metachromatic material which was faintly PAS positive was found only in the retentate of fraction 3. *E*, Electrophoretic pattern of serum stained with coomassie blue.



amine in the presence of UDP-N-acetyl-galactosamine and a polypeptidyl:N-acetyl-galactosamine transferase from bovine submaxillary glands⁸. N-Acetyl-galactosamine is present in blood group A substance, and although CEA contains no N-acetyl-galactosamine it does show blood group A-like activity^{9,10}. N-Acetyl-glucosamine is a characteristic constituent of CEA⁹. Perchloric acid extracts of colonic carcinoma contain cationic proteins that do not bind to DEAE 'Sephadex' (unpublished data). That cationic proteins may also be present in homogenates of colonic carcinomata is suggested by the observation that human, malignant cell lines have on their surface¹¹ an antigen that is immunologically related to basic proteins which can be extracted from these cell lines and from human brain and malignant tissue by treatment with HCl at pH 2.5¹¹⁻¹³. CEA specificity could therefore be formed during the homogenization of carcinomata by glycosyl transferases coupling carbohydrate residues to sites that are not exposed in normal tissues. Again, it may be pertinent to draw attention to the observation that low molecular weight (about 5,000) glycopeptides form a major fraction of cell membranes and can be extracted with either sodium dodecyl sulphate or formic acid¹⁴. Polymerization is a characteristic of these glycopeptides and lyophilization results in aggregates that cannot be readily dissociated¹⁴. CEA is a component of the cell surface¹⁵.

Because of the unusual characteristic of the cationic protein present in CEA, it was considered that these preliminary observations might be of particular interest to workers using CEA preparations in radioimmunoassay and other immunological tests.

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N. R. HUGHES

The New South Wales State Cancer Council,
Special Unit, Prince of Wales Hospital,
Randwick, NSW 2031

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Receptor for Monomeric IgM on Guinea-pig Splenic Macrophages

It is now well established that an optimal immune response resulting in the production of antibody to most antigens requires interaction between three distinct cell types: T (thymus-derived) and B (thymus-independent) lymphocytes¹, and macrophages². Studies to elucidate the mechanism of interaction or collaboration have implicated soluble factors released by T cells which include both non-specific components³⁻⁶ and antigen-specific components⁶⁻⁹ which are bound by macrophages^{8,9}. IgM has been demonstrated in T cell membranes^{10,11} in monomeric form¹¹. Antigen binding by T cells, as judged by rosette formation¹² or specific helper function at low doses of antigen¹³, can be inhibited by antibody to IgM determinants. Evidence suggests that the specific collaborative factor released by T cells consists of antigen complexed with monomeric IgM, which becomes bound to the surface of macrophage-like cells (refs. 9, 14 and 15 and E-P. Rieber and G. Reithmüller, submitted for publication). Macrophage binding of antigen specific factors released by T cells has also been shown in studies on the killing of allogeneic tumour cells *in vitro*¹⁶. Here I show that a receptor for monomeric IgM is present on macrophages from guinea-pig spleen. Pentameric (19S) IgM is not bound by these cells to any significant extent. Splenic macrophages were defined by the following characteristics: (1) rapid uptake of neutral red stain; (2) ingestion of carbonyl iron particles; (3) presence of a receptor for the Fc portion of guinea-pig γG_2 ; (4) uptake *in vivo* of alum precipitated bovine serum albumin (BSA).

My data, summarized in Tables 1 and 2, show that approximately 1% of spleen cells possess a receptor which is specific for the Fc portion of IgG₂ but not for IgM. Such a receptor has been shown to be characteristic of guinea-pig alveolar¹⁷ and peritoneal macrophages^{17,18}. Rosette forming cells (RFCs) were also found to stain rapidly with neutral red (Table 2). Approximately 50% of stained cells had the appearance of macrophages, the remainder consisted of

Table 1 Rosette Formation on Normal and Macrophage-depleted Spleen Cells using IgG* and IgM†-sensitized Red Cells

Rosette forming cells per 10 ³ spleen cells ‡		
IgM-coated SRBCs plus normal spleen cells	IgG-coated SRBCs plus normal spleen cells	IgG-coated SRBCs plus macrophage depleted spleen cells
1.0	15.0	6.0
1.5	12.0	4.5
1.3	8.5	5.5
0.6	15.5	5.5

* Concentration, 0.25 mg ml⁻¹.

† Concentration, 0.5 mg ml⁻¹.

‡ Mean sample size = 3.5×10^3 spleen cells.

Splenic tissue was minced and irrigated with Hanks balanced salt solution (Hanks BSS) pH 7.2. Cells were separated from tissue fragments by differential centrifugation and freed of erythrocytes by differential hypotonic lysis and agglutination.

Splenic cells were subjected to a rosette formation procedure using sheep red blood cells (SRBCs) sensitized with guinea-pig anti-SRBC antibody of either the γG or γM class (twice separated on 'Sephadex' G200). The degree of coating with 19S and 7S antibody was comparable as judged by haemagglutination using rabbit anti-guinea-pig serum.

0.1 ml of a 50% suspension of SRBCs was incubated with 0.5 ml of antibody at the concentration stated, in Hanks BSS pH 7.2 for 90 min at 37° C. The cells were then washed twice and resuspended as a 20% suspension. Rosette formation was carried out by mixing 0.1 ml of this suspension with 0.1 ml of a suspension of nucleated spleen cells containing 10⁷ cells. The mixture was centrifuged at 220g for 5 min at room temperature and resuspended by gentle aspiration in a Pasteur capillary pipette repeated twelve times. The cells were examined under phase contrast illumination at $\times 600$. Nucleated cells completely surrounded by ten or more SRBCs were scored as rosettes.

Table 2 Representative Table: Characterization of Receptor-specificity and Receptor-bearing Cells

IgG rosettes Pretreatment ‡	Positive cells per 10 ³ spleen cells		Antigen uptake †	
	Neutral red staining* Pretreatment		Pretreatment	
None	8.2	None	None	6.0
IgG ₁	5.9	None	None	5.6
IgG ₂	0.7	None	None	5.3
Fab	8.4	None	None	
Fc	0.7			

* Spleen cells at a concentration of 5×10^7 cells ml⁻¹ were stained in a 0.002% solution of neutral red. At room temperature receptor bearing cells stained rapidly (2–5 min).

† 0.5 ml of fluorescein-conjugated BSA at 20 mg ml⁻¹ precipitated on alum was injected intravenously and spleen cells were extracted after 2 h. Following rosette formation with IgG-sensitized SRBCs, RFCs were examined for fluorescence in ultraviolet illumination.

‡ Spleen cells at a concentration of 7.5×10^7 cells ml⁻¹ were incubated with IgG subclass preparations at 6 mg ml⁻¹ or IgG₂ molecular fragments at 1 mg ml⁻¹ for 30 min at room temperature. The cells were centrifuged down and resuspended in fresh Hanks BSS.

Table 3 Rosette Formation on Normal and Macrophage-depleted Spleen Cells using SRBCs Sensitized with Pentameric (19S) IgM or Monomeric (7S) IgM

Rosette forming cells per 10 ³ spleen cells*			
SRBCs coated with 19S IgM at a concentration of:		SRBCs coated with monomeric IgM at a concentration of:	
1 mg ml ⁻¹	0.5 mg ml ⁻¹	0.5 mg ml ⁻¹	0.5 mg ml ⁻¹ Macrophage depleted spleen cells
Normal spleen cells	Normal spleen cells	Normal spleen cells	
3.0	1.0	8.1	3.2
2.75	1.5	8.0	3.2
2.2	1.7	4.5	2.9
2.5	1.5	7.6	3.4
		7.8	3.0

* Mean sample size = 4×10^3 .

Monomeric IgM was prepared by reduction and alkylation of IgM obtained from guinea-pig and anti-SRBC antisera. Dithiothreitol (DTT) at a concentration of 0.7 mM was incubated with IgM at 2.5 mg ml⁻¹ in 100 mM Tris HCl pH 8.0 for 1 h at room temperature. Reduction was stopped by addition of a four-fold molar excess of iodoacetamide and the solution was dialysed against Hanks BSS pH 7.2 for 18 h. The optimal concentration of DTT in this procedure was determined using a range of DTT solutions (0.05–1.5 mM). Thus, after an 18 h dialysis the reduction products were analysed by polyacrylamide disc electrophoresis in the presence of sodium dodecyl sulphate²⁵. The major products obtained using 0.5–1.0 mM concentrations of DTT were monomeric IgM (IgMs) and a half molecule consisting of one heavy and one light chain (H-L fragment). Traces of trimer and dimer were detectable using 0.6 mM DTT.

SRBCs were specifically coated with monomeric IgM and possibly H-L fragments²⁶ by incubation for 90 min at 37° C in a solution containing reduction products at a concentration of approximately 1 mg ml⁻¹. Red cells were then washed once in a 20-fold volume of Hanks BSS and resuspended as a 20% suspension. Rosette formation on spleen cells using SRBCs sensitized in this way was compared with rosette formation using SRBCs sensitized with pentameric (19S) IgM. Two controls were adopted, one using SRBCs coated with IgM at 0.5 mg ml⁻¹ (the approximate concentration of monomeric IgM in the sensitizing solution) and the other using IgM at 1 mg ml⁻¹ (the concentration of the total reduction products: IgMs + H-L).

Magnetic depletion of macrophages together with some monocytes was achieved by incubating spleen cells at a concentration of $15\text{--}20 \times 10^6$ cells ml⁻¹ with carbonyl iron powder (type SF, GAF Gt. Britain Ltd, Manchester) at a concentration of 3 mg ml⁻¹ in Hanks BSS pH 7.2, for 30 min at 37° C with intermittent mixing. Incubation was carried out in tubes coated with dimethyldichlorosilane and macrophages were precipitated by repeated exposure of the suspension to a magnetic field.

monocytes and Kurloff cells¹⁹. Macrophages together with some monocytes were selectively depleted using carbonyl iron and magnetic precipitation (Table 1). The uptake of alum precipitated BSA *in vivo* was also found to be a property of receptor-bearing macrophages (Table 2).

Results of rosette formation are presented in Table 3.

Significant binding was found only in the monomeric IgM preparation. RFC were identified as macrophages by magnetic depletion following carbonyl iron ingestion. Thus macrophages in guinea-pig spleen, previously characterized by the four properties described, also bind monomeric IgM specifically combined with SRBC determinants. Binding occurs in the absence of complement and lymphocytes do not show binding activity; pentameric (19S) IgM is not bound to any significant extent. The receptor may also be present on monocytes which are not depleted by the carbonyl iron procedure.

The hinged nature of pentameric IgM^{20,21} may allow attachment of all combining sites to red cell determinants. The ratio of monomeric to pentameric molecules on equivalent areas of red cell surface, however, is unlikely to be greater than 5:1 and in antibody excess will be less than this so that it is not an increase in the number of Fc μ regions per unit surface area which facilitates binding of monomeric IgM.

The receptor for monomeric IgM on splenic macrophages may be involved in the collaborative function of such cells in the immune response^{9,14,15}. Feldmann has suggested that the binding by macrophage-like cells of monomeric IgM/antigen complexes released by T cells may facilitate the presentation to B cells of antigenic determinants in an immunological array⁹. The model suggests a possible regulatory effect in T-B cell cooperation in terms of saturation of macrophage receptor sites and could account for antigenic competition at the level of availability of receptors for monomeric IgM²². Any equation between reductive monomer and T cell IgM must be made with caution, however^{15,23}. Another area in which a macrophage receptor for monomeric IgM might be involved is in the specific cytotoxic arming of macrophages by antigen specific factors released by T cells¹⁶.

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JOHN RHODES*

Department of Zoology,
University College,
Gower Street,
London WC1E 6BT

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* Present address: Laboratory of Clinical Investigation, Building 10, Room 11B-13, National Institutes of Health, Bethesda, Maryland 20014.

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Cocrystallization of Proinsulin and Insulin

THE occurrence of small amounts of proinsulin in crystalline preparations of bovine, porcine, and rat insulin¹⁻⁴ and the many similarities in properties of proinsulin and insulin prompted us to consider the possibility that proinsulin cocrystallizes with insulin. Spectral⁵ as well as immunological⁶ studies have provided evidence that the insulin moiety in proinsulin has a very similar conformation to that of insulin, and proinsulin exhibits the same tendency to form dimers in acidic solution and higher polymers, mainly hexamers, in neutral solutions containing zinc ions⁷. Studies on crystals of proinsulin by Low and coworkers⁸ have confirmed the presence of dimers in the unit cells of these crystals. All these observations suggest that the connecting polypeptide segment in proinsulin is oriented away from those surfaces involved in dimer and hexamer formation.

Hexameric structures stabilized by two zinc atoms have been shown by the recent work of Hodgkin and her coworkers⁹ to form the unit cell in porcine zinc insulin crystals. The X-ray diffraction studies also have revealed that the carboxyl terminal region of the B chain and the amino terminal region of the A chain are located about 8 Å apart, near the external or peripheral surface of the insulin monomers within the hexamers. These locations would allow

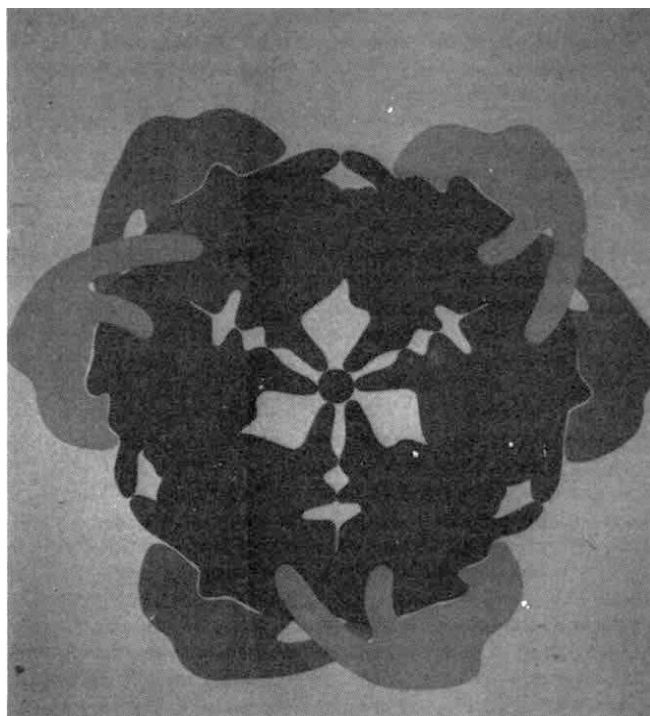


Fig. 1 Hypothetical representation of a proinsulin hexamer viewed along the three-fold axis of the hexamer. The central density represents the two zinc atoms which coordinate to the six histidine side chains at position 10 in the B chain. The densities surrounding the zinc atoms and extending towards them represent the 6 insulin monomers outlined according to the data of Hodgkin and coworkers⁹. The connecting polypeptide is shown in lighter grey around the peripheral portion of the insulin hexamer and the sites of attachment to the insulin chains are indicated by the arms.

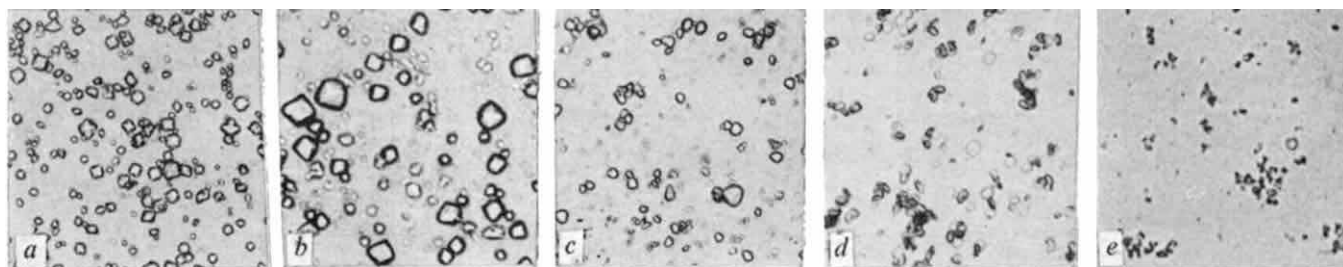


Fig. 2 Photographs of crystals obtained with various proportions of proinsulin and insulin. *a*, Insulin, $\times 120$; *b*, 8% proinsulin, $\times 120$; *c*, 17% proinsulin, $\times 120$; *d*, 33% proinsulin, $\times 250$; *e*, 50% proinsulin, $\times 250$.

the connecting polypeptide to extend into the space overlying those regions of the insulin monomer that are normally exposed to the solvent in solution. Whether the connecting peptide has a defined, three-dimensional arrangement over this region of the insulin moiety of proinsulin is a question that has not yet been resolved, although optical studies have not provided evidence for the existence of defined secondary structure in this part of the molecule. A hypothetical arrangement of a proinsulin hexamer is illustrated in Fig. 1.

If the structure shown in Fig. 1 is correct in principle, then it is easy to visualize the replacement of individual proinsulin molecules within such a hexamer with insulin molecules. Conversely, in insulin hexamers, an occasional molecule of proinsulin might replace an insulin monomer. Of course, in the presence of a relatively large proportion of proinsulin (<5–10%), the connecting polypeptides would undoubtedly interfere with the packing of hexamers into crystals. Low proportions of proinsulin might, however, be well tolerated within insulin crystals and this could account for its tendency to be retained through repeated recrystallizations of the insulin.

To test this hypothesis, we attempted crystallization of mixtures of bovine proinsulin and insulin in various proportions corresponding on a molar basis to 0.5, 1.0, 2.0 and 3.0 mol proinsulin/6 mol total of insulin and proinsulin. Crystallization was carried out in 0.095 M sodium citrate buffer (pH 6.0) containing 0.03 M NaCl, 0.012 M ZnCl₂ and 16% acetone (v/v) at room temperature or at 5° C. Initial protein concentration was 0.33 $\mu\text{mol ml}^{-1}$ in all cases. There was a striking correlation between the amount of proinsulin added to the mixture and the time required for the first visible signs of crystallization. Thus insulin alone began to crystallize within 5 min. With 8% proinsulin crystallization required 20–30 min; with 17%, several hours were required; with 33%, 1–2 days, and with 50% approximately 1 week was required at 5° C before very small crystals began to appear.

Photographs of the crystals are shown in Fig. 2. In the samples containing 8 or 17% proinsulin, rhombohedral crystals were formed but these differed from those of pure insulin in having significantly rounded borders. No normal crystals were observed although there was great variability in size, some being quite large. With 33% proinsulin, the crystals were smaller and in many instances were so distorted that they resembled flattened, round disks suggestive of erythrocytes. Some rounded rhombohedral forms were also evident but no normal crystals could be seen. With 50% proinsulin, very fine crystals were obtained which were either rounded or so small that definite characteristics could not be ascertained. No new crystalline form appeared even after storage for more than 30 days.

The tubes containing the crystals were centrifuged and the mother liquor was separated from the crystals which were washed once with fresh cold crystallization solution. The crystals were then dissolved in Tris-glycine buffer (pH 8.6) and aliquots were subjected to polyacrylamide electrophoresis at pH 8.9 (Fig. 3). The stained gels were scanned with a Schoeffel Instrument Corp. Model SD-3000 densito-

meter. By comparison with stained standards of proinsulin and insulin, it could be shown that in each case the proportions of proinsulin and insulin in the crystal corresponded well with those in the initial mixtures without showing any apparent deviation even in the samples containing 33% proinsulin (the 50% crystals were not analysed).

These results confirm the hypothesis that proinsulin can co-crystallize with insulin. Proinsulin itself does not crystallize under these conditions and no indications were seen of the presence of different kinds of crystals in these preparations, so it seems very likely that the composition of the crystals with respect to proinsulin and insulin was relatively uniform throughout. Similarly, the inhibition of crystallization in the mixtures containing a high proportion of proinsulin suggests that the association properties of proinsulin are closely similar to those of insulin. Thus, presumably, mixed dimers and higher polymers form relatively rapidly, but because of the lack of symmetry these tend to pack more slowly into crystals. It is possible that by systematically varying the crystallization conditions, a new mixed crystal form of proinsulin and insulin might be found, and such forms might lend themselves to the elucidation of the three-dimensional structure of proinsulin. The ability of proinsulin to efficiently cocrystallize with insulin provides a convenient and powerful tool for isolating small amounts of proinsulin and related intermediate structures from pancreatic extracts by crystallization with the insulin. This method proved valuable for isolating small amounts of human proinsulin¹⁰.

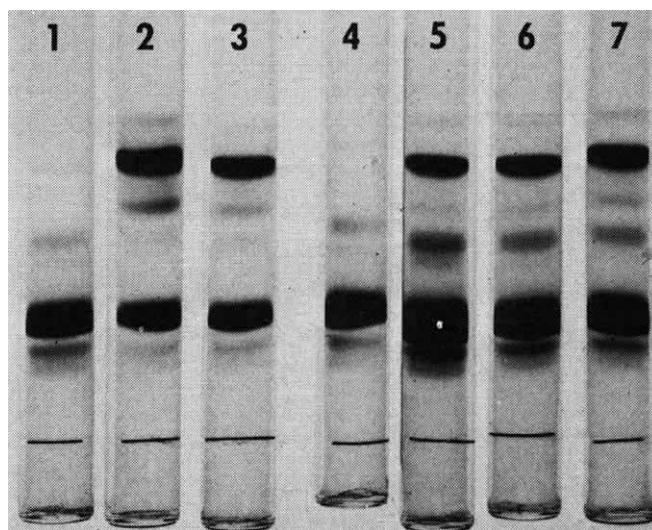


Fig. 3 Photographs of polyacrylamide gel electrophoretograms stained with amido schwarz. Anode is below, proinsulin is the major slow migrating component. The gels were loaded as follows: (1) standard of insulin, 80 μg ; (2) standard of insulin, 40 μg , and proinsulin, 60 μg ; (3) standard of insulin, 40 μg , and proinsulin, 30 μg ; (4) insulin crystals; (5) 8.3% proinsulin crystals (measured value: 9%); (6) 16.7% proinsulin crystals (measured value: 17.5%); (7) 33.3% crystals (measured value: 32%).

It may be anticipated that cocrystallization may occur between many precursor and product peptides, and among other closely related proteins. Moreover, the ability of proinsulin to form hexamers in which the connecting polypeptide is on the external exposed surfaces suggests a possible role for these structures in regulating the conversion of proinsulin to insulin and protecting the newly formed insulin during its biosynthesis within the pancreatic beta cells. Particularly the retarding of crystallization by proinsulin may prevent the premature formation of crystalline granule inclusions¹¹ before the conversion of most of the proinsulin has occurred.

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D. F. STEINER

Department of Biochemistry,
University of Chicago,
Chicago, Illinois 60637

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Functional Preservation of Canine Heart-Lung Preparations by Perfusion with Pure Haemoglobin Solution

THIS study deals with classic *ex-vivo*, self-perfusing heart-lung preparations, in which the circulating medium is a pure human haemoglobin solution free of stroma. Although this scheme was first devised for preservation for subsequent transplantation, it also provides a convenient method of testing the effects of pharmacological agents on such preparations without interference from the figured elements of the blood for periods up to 6 h.

The haemoglobin solution was prepared by lysis of out-dated packed erythrocytes in hyposmolar phosphate buffer, ultracentrifugation, and microfiltration through a series of filters down to a pore size of 0.22 μ m. Electrolyte balance similar to that of human plasma was achieved by dialysis through a Kolff twin coil artificial kidney against a standard dialysing fluid adjusted to pH 7.4. All procedures but the last were carried out at 4° C¹⁻⁴. Mongrel dogs were heparinized (2 mg kg⁻¹) and killed with an overdose of 'Pentothal'. The thorax of each animal was opened by an incision which

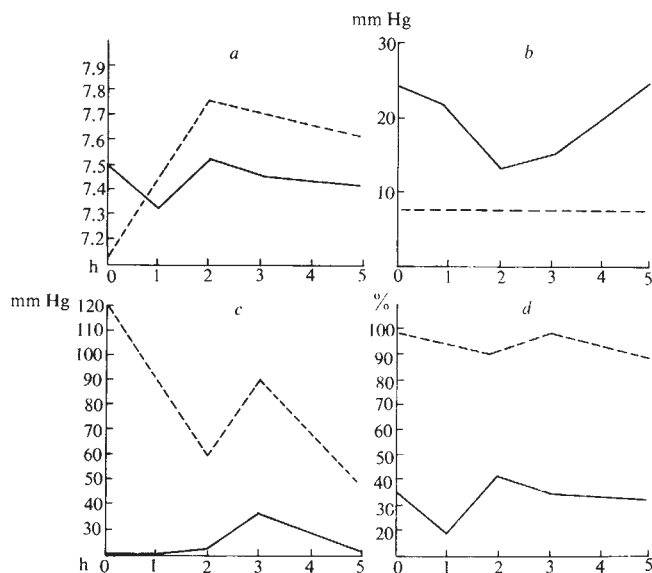


Fig. 1 Variations of a, pH, b, pCO₂, c, pO₂, and d, haemoglobin oxygen saturation in whole canine blood and human haemoglobin solution during functional preservation of self-perfusing heart-lung preparations. —, Fresh heparinized whole canine blood; ---, human haemoglobin solution.

split the sternum. With the lungs being ventilated mechanically, the azygos vein, venae cavae, brachiocephalic artery and thoracic aorta were clamped. The subclavian artery was cannulated and connected to a reservoir filled with perfusate at 37° C. The heart beat was restored by gentle massage and defibrillation. The heart-lung preparation, now self-perfusing, was dissected out and transferred to a homeostatic chamber where it was partially immersed in a solution of balanced electrolytes at 37° C doubling as hydraulic support⁵.

In six experiments, the human haemoglobin solution was used as the perfusate. The blood trapped in the heart and lungs was flushed through the brachiocephalic artery, the haemoglobin solution being, therefore, the only medium within the cardiopulmonary circulation. Controls consisted of six identical preparations perfused with heparinized blood obtained by previous exsanguination of the experimental animal into the reservoir.

In all cases, the pressure in the brachiocephalic artery was monitored continuously and samples of blood or haemoglobin were taken from the vessel at regular intervals for pH, pCO₂, pO₂, and haemoglobin oxygen saturation determinations. As progressive seepage from the preparations decreased the volume of the reservoir, blood or haemoglobin solution were added but no nutrients or insulin. Experiments were terminated when the heart went into irreversible fibrillation or when the available supply of blood or haemoglobin was exhausted. Histological examination was performed on all preparations.

"Survival" times for the haemoglobin-perfused preparations ranged from 2 to 6 h (average 3.5), while the blood-perfused controls lasted from 5 to 12 h (average 8.25). Both experimental and control arterial pressures were comparable and remained steady until the end, which was usually preceded by an abrupt fall in pressure.

From Fig. 1, which gives the average values for pH, pCO₂, pO₂, and haemoglobin oxygen saturation during the first 5 h in each group, it can be seen that haemoglobin oxygen saturation remained at 90–99% throughout in the experimental group, but dropped within the 30–45% range in the control group. The preparations perfused with human haemoglobin tended to become markedly alkaline, but when fresh solution was added to the reservoir, there was a sharp drop in pH: for instance, in one experiment, from 7.90 to 7.40 (the pH of the fresh solution), followed by a rise to a final value of 7.80. A marked rise in pO₂ also accompanied the addition of fresh

solution (Bohr effect) while $p\text{CO}_2$ remained low. In contrast, with the same rate of mechanical ventilation, the preparations perfused with canine blood were less alkaline and the addition of fresh blood resulted in smaller variations of blood gases and pH parameters.

Examination of the tissue specimens after the end of the perfusion showed the lungs of the control preparations to be oedematous and in some cases haemorrhagic, while oedema and haemorrhage were absent in the haemoglobin-perfused preparations. In addition, subendocardial haemorrhages were larger and more frequent in the blood-perfused hearts.

The maintenance of myocardial contractility and normal arterial pressure suggests that these preparations retain their ability to sustain the circulation of the intact animal. Preparations similarly preserved in another series of experiments have been transplanted successfully into suitable recipients and have supported their circulatory load for periods averaging 6 h^{5,6}.

That the solution adequately oxygenates the perfused tissues is further demonstrated by the high level of oxygen saturation at which it remains during the course of these experiments: a finding of particular interest, especially when compared with the behaviour of the whole blood in the control perfusions. The sharp fluctuations in pH and $p\text{O}_2$ observed in the haemoglobin-perfused preparations probably relate to the continued metabolism of the heart and lungs. In the absence of kidneys, this leads to severe alkalosis, because the haemoglobin solution has distinctly less buffering capacity than whole blood.

Oedema formation has marred the field of organ perfusion ever since its inception and has been familiar to early⁷ as well as to later workers⁸. Increasing vascular resistance, decreasing flow rate, and progressive swelling have been seen as a reflexion of injury to the endothelial lining caused by small corpuscular elements. Addition of a glass wool microfilter in a heart perfusion circuit using whole blood has increased "survival" times⁹. A non-dialysable toxin or unrecognized metabolite has, however, also been implicated¹⁰. Protection of the capillary endothelium was therefore sought in various ways and organ preparations perfused with platelet-rich plasma¹¹ and the use of adjuvants such as dexamethasone¹² have yielded notable results.

These considerations certainly suggest lines for further research but do not allow more than a tentative conclusion. The pathological changes observed in our blood-perfused preparations may be caused by stromal elements of the blood damaging to endothelial walls, whereas the less severe changes seen in the haemoglobin-perfused preparations could be secondary to loss of cell membrane integrity as caused by increased metabolic toxins or corticoid depletion. In any case, isolated canine heart-lung preparations perfused with a pure haemoglobin solution represent a stable system the pharmacokinetic potentialities of which seem worthy of investigation.

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W. M. RIEDESEL II
I. ERDAMAR
S. J. DOS

Department of Surgery,
Cornell University Medical College,
1300 York Avenue,
New York, NY 10021

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Anaerobic, Glycogen-dependent Transport of Amino Acids by the Placenta

THE concentration of amino acids is greater in foetal than in maternal blood and their placental transport is fairly well established to depend on energy dependent processes¹. Although it is reported that placental transport of amino acids occurs only in aerobic conditions²⁻⁴, we show here that the human placenta can accumulate amino acids anaerobically against a concentration gradient, and that this is sustained by the utilization of glycogen.

Accumulation of α -aminoisobutyric acid (AIB), a non-metabolizable amino acid, was measured in slices of near-term human placental tissue in control conditions and in the presence of metabolic inhibitors. Placental slices, about 0.3 mm thick and weighing a total of 300 mg, were placed in 50 ml vessels containing 10 ml balanced Krebs saline, pH 7.3. After a period of preincubation designed to produce a steady state of tissue electrolytes, the slices were transferred to another flask containing buffered saline plus 0.1 mM ¹⁴C-AIB, with or without 0.1 mM dinitrophenol (DNP) or 0.1 M sodium arsenate and gassed with 100% O_2 or N_2 . Preincubation and incubation were carried out at the same temperature, either 25° or 37° C. After varying times the slices were removed, blotted, weighed and homogenized in 4 ml of cold 10% trichloroacetic acid. After centrifugation, aliquots of the supernatant were counted in a liquid scintillation counter. Calculations of the intracellular to extracellular accumulation ratio ($[\text{AIB}]_i/[\text{AIB}]_o$) and uptake rate (V) at 1 h in μmol per (ml intracellular water $\times$ h) were made after correcting for extracellular and total water spaces (see, for example, ref. 4). The ratio $[\text{AIB}]_i/[\text{AIB}]_o$ expresses the degree of intracellular accumulation. Values greater than 1.0 indicate transport against a concentration gradient. Placental glycogen concentration was determined using a modification of Montgomery's method⁵.

Results of placental AIB accumulation at 25° C after 45 min preincubation and as a function of time in control conditions, with anaerobic atmosphere (100% N_2), DNP or arsenate, are shown in Fig. 1. Control AIB accumulation was almost linear during the first 3 h of the experiment. In the presence of N_2 or DNP, an inhibitor of electron transport in mitochondria, accumulation was decreased about 20 and 40%, respectively, but was still significantly greater than 1. (DNP concentrations of 0.05 to 0.2 mM inhibited the system to essentially the same extent indicating that 0.1 mM DNP was maximally inhibiting.) In contrast, inclusion of arsenate, an inhibitor of glycolysis, and thus of anaerobic metabolism, resulted in an accumulation ratio of about 1, a value indicating diffusion equilibrium. It should be noted that optimal AIB accumulation in anaerobic conditions depended on minimal time delay between delivery and the start of the experiment. AIB uptake rates in $\mu\text{mol}/(\text{ml} \times \text{h})$ were: control = 0.18 (± 0.06); N_2 = 0.14 (± 0.03); DNP = 0.10 (± 0.02).

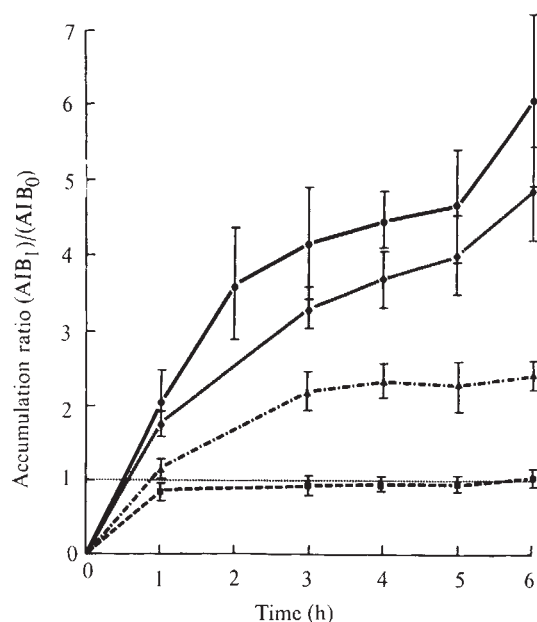


Fig. 1 Ratio of concentration of α -aminoisobutyric acid (AIB) in free water of placental tissue to its concentration in the media, as a function of time at 25° C following 45 min preincubation. Control (●); 100% N₂ atmosphere (◆); 10⁻⁴ M dinitrophenol (▲); 10⁻¹ M sodium arsenate (■). An accumulation ratio of 1 indicates diffusion equilibrium. Each point is the mean of nine determinations on these animals; bars represent 1 s.d.

That glycogen is the energy source for the accumulation is suggested by an increased rate of disappearance of this storage polysaccharide in anaerobic conditions. Placental glycogen concentration was about 27% lower than control values both 1 and 2 h after incubation of the slices simultaneously in N₂, DNP and 0.055 mM adrenaline.

If the tissue slices were preincubated at 37° C for 3 h under N₂ and in the presence of DNP and epinephrine, so as

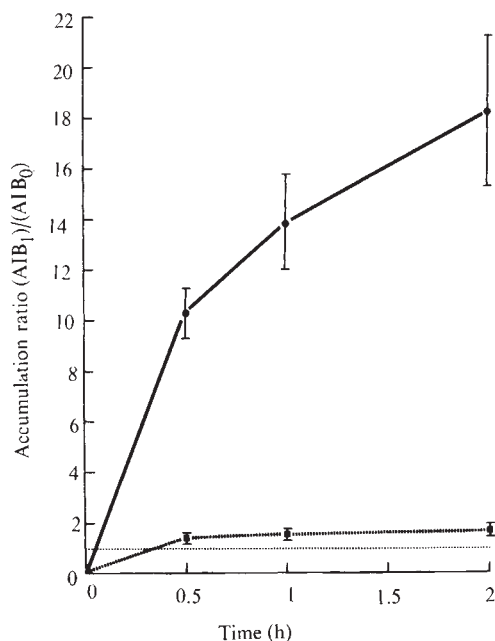


Fig. 2 Accumulation ratio for AIB at 37° C after a 3 h preincubation without (●) and with (■) simultaneous N₂ atmosphere, 10⁻⁴ M DNP and 5.5 × 10⁻⁵ M adrenaline to decrease available energy source. Each point is the mean of nine determinations on these animals; bars represent 1 s.d.

to remove or considerably reduce available glycogen, anaerobic AIB accumulation was nearly abolished (Fig. 2). Control accumulation ratios were considerably higher after a 3 h preincubation than after a 45 min preincubation (compare Figs. 1 and 2). This phenomenon, which is not understood at present, seems to be only partly due to the higher temperature and is under investigation.

That the lower accumulation ratio is truly due to the removal of the energy source and not to cell death is shown by the addition of glucose to the tissues after anaerobic preincubation to reduce available glycogen (Fig. 3). In the presence of 10 mM glucose, the slices can once again accumulate amino acids anaerobically (Fig. 3), whereas accumulation was minimal in the absence of glucose.

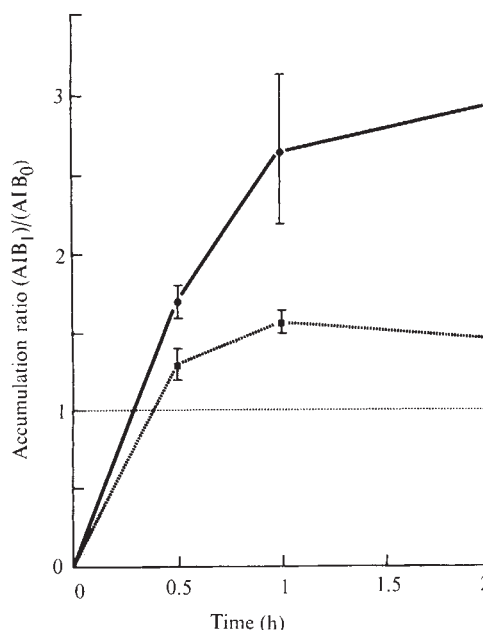


Fig. 3 Accumulation ratio of AIB at 37° C after 3 h preincubation with simultaneous N₂ atmosphere, 10⁻⁴ M DNP and 5.5 × 10⁻⁵ M adrenaline. In the presence of added 10 mM glucose (●) the accumulation ratios were significantly greater than when glucose was not added (■). Each point is the mean of nine determinations on three animals; bars represent 1 s.d.

Sybulski and Tremblay² reported that N₂ and 1.3 mM DNP decreased glycine uptake by human placental slices about 27% and 38% respectively. Whether or not accumulation against a concentration gradient occurred cannot be determined from their data. Litonjua *et al.*³ also reported decreased AIB uptake by human placental slices under 100% N₂ with no accumulation noted. Dancis *et al.*⁴ reported that 0.2 mM DNP and 10 mM sodium cyanide decreased [AIB]_i/[AIB]_o about 34% and 62% respectively in 1 h from a control value of about 2.25. The actual values were not given and presumably the accumulation ratios were 1 or less.

Compatible with our results is the finding of Reynolds and Young⁶ that the rate of α -amino acid nitrogen transfer in the *in situ* perfused guinea-pig placenta was not decreased when maternal arterial O₂ tension was lowered to 30 mm Hg, or by the addition of either 0.5–5 mM DNP or 2–10 mM KCN to the perfusate. Whether active accumulation was affected by these procedures was not noted.

Although all tissues possess the glycolytic pathway and therefore have the potential for supporting energy-dependent processes by anaerobic metabolism, anaerobic amino acid transport does not occur in adult intestine⁷⁻⁹ or kidney¹⁰.

On the other hand, foetal tissues have an unusual propensity to function independently of oxidative phosphorylation compared with adult tissues¹¹. Foetal and newborn rabbit small intestine anaerobically transports L-histidine, L-tyrosine and 6-deoxy-D-glucose⁸. Butt and Wilson⁹ showed that embryonic guinea-pig yolk sac and intestine transport amino acids anaerobically. Whittam¹² reported anaerobic maintenance of Na⁺, K⁺ and water concentrations in immature and newborn rabbit and rat kidney cortex slices. Near term foetal liver slices have been shown¹³ to carry out lipogenesis anaerobically more rapidly than adult liver. Term placental slices continue to utilize fructose and glucose after 1 h of complete anoxia¹⁴.

Our studies suggest that glycogenolysis can support placental transport in anaerobic conditions. Supporting this, Demers *et al.*¹⁵ reported that DNP and severe hypoxia decreased the glycogen content of placental villi in organ culture with a shift from glucose-6-phosphate-dependent to independent glycogen synthetase (the enzyme catalysing glycogen synthesis), and a concurrent activation of glycogen phosphorylase (a glycogen-degrading enzyme).

To what extent anaerobic transport serves as an adaptation to increase foetal survival during periods of acute hypoxia cannot be stated. It would seem that the foetal brain would suffer irreversible damage before the foetus suffered from amino acid deficiency. Perhaps the ability to function anaerobically has survival value for the foetus at an early period in gestation; for example, before development of the central nervous system is complete. In support of this, glycogen utilization by human placenta occurs at a substantially greater rate during the first third of gestation than thereafter¹⁶.

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Note added in proof. Smith *et al.*¹⁷ have also shown recently that the enhancement of placental AIB accumulation is a function of prolonged pre-incubation.

LAWRENCE D. LONGO
PETER YUEN
DAVID J. GUSSECK

*Departments of Physiology and Biochemistry,
School of Medicine,
Loma Linda University,
Loma Linda, California 92354*

Received February 12, 1973.

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Complementation Analysis of Maple Syrup Urine Disease in Heterokaryons derived from Cultured Human Fibroblasts

MAPLE syrup urine disease (MSUD) is an autosomal recessive disorder characterized by keto acidosis, convulsions, mental retardation and a maple syrup odour in the urine. The clinical phenotype varies among different families, with a severe early onset in the classical form of the disease and milder symptoms with delayed onset in variants. The biochemical defect is an inability to degrade the three branched-chain amino-acids (BCAA), leucine, isoleucine, and valine, as a result of a deficiency in decarboxylation of the branched-chain α -keto acids (BCKA). The enzyme defect is expressed in skin fibroblast cultures derived from affected patients, and the level of residual BCKA decarboxylase activity correlates with the severity of clinical symptoms. The heterogeneity in clinical presentation and the level of residual decarboxylase activity appear to be genetically determined¹.

Intergenic^{2,3} and possibly interallelic^{4,5} complementation of other mutations has been reported in fused mammalian cells. We approached genetic heterogeneity in MSUD by using Sendai virus-induced fusions of skin fibroblasts derived from unrelated patients. If fusing two different mutant cells corrects their common BCKA decarboxylase deficiency it is assumed that they have different genetic lesions. In MSUD the efficiency of complementation is high in certain heterokaryons and can be detected without a selective system. The ability of MSUD fibroblasts to complement one another bears no apparent relationship to the severity of the disease or the level of residual BCKA decarboxylase activity.

Fibroblast strains derived from skin biopsies were grown in Waymouth's medium containing 15% foetal calf serum and antibiotics⁶. The severity of MSUD in the patients from whom fibroblasts were derived was classified on the basis of clinical phenotype, dietary tolerance for BCAA, and residual BCKA decarboxylase activity in fibroblast cultures¹: BaKu and TeHe—grade 1: classical MSUD (0–2% of normal enzyme activity). NiRo—grade 2: variant MSUD (2–8% of normal enzyme activity). ElHa—grade 3: variant MSUD (8–15% of normal enzyme activity).

BCKA decarboxylase was assayed in intact cell suspensions rather than cell lysates because BCKA decarboxylase activity is greatly reduced in disrupted normal or mutant fibroblasts. The branched-chain amino-acids, labelled in the C-1 position, were used as substrates because lower and more consistent blanks were obtained with them than with the branched-chain α -keto acids. The first degradative step, transamination, is not rate limiting in normal or MSUD subjects⁷. BCKA decarboxylase activity of the cells is expressed as the ratio of radioactive CO₂ evolved to the amount of radioactivity incorporated into protein (TCA precipitated material), the latter serving as a measure of the metabolically active cells^{1,7}. Within each experiment the total number of cells per flask, total cell protein per flask, and the incorporation of radioactive BCAA into TCA precipitated material was similar for replicate cell mixtures with and without added Sendai virus. Increased ratios of CO₂ to protein, such as those observed in complementing cell pairs treated with virus, were due to increased evolution of radioactive CO₂ rather than selective reduction in protein synthesis.

The method of Velasquez *et al.*⁸ yielded the greatest number of heterokaryons and the best recovery of viable cells. The number of nuclei per cell varied from two to as many as six or seven, but most of the fused cells had either two or three nuclei. For complementation studies the hybridized cells were maintained in the original confluent monolayers, thus few cell divisions occurred after cell fusion. Nuclear fusion was not observed in our preparations.

Table 1 shows the result of studying confluent cultures of

fibroblasts grown from single individuals. Multinucleated cells were 15–30% of the total cell number in virus treated flasks, but the BCKA decarboxylase activity of these flasks was not significantly different from that of untreated flasks

Table 1 Effect of Inactivated Sendai Virus on BCKA Decarboxylase Activity in Homokaryons

Cell line	Diagnosis	BCKA decarboxylase activity *		Complementation ratio †	
		Control cells Individual flasks (Average)	Fused cells Individual flasks (Average)		
MuJa	Normal	179.4	145.7	1.1	
		150.2 (164.8)	170.5 (187.2)		
GaSa	Normal (Exp. 1)	158.5	148.5	1.0	
		163.8 (170.6)	172.1 (167.0)		
	(Exp. 2)	189.4	180.3	1.0	
		404.5 (395.5)	414.2 (386.4)		
BaKu	Classical	0	0	(1)	
		0 (0)	0 (0)		
		0	0		
TeHe	Classical	0	0	(1)	
		0 (0)	0 (0)		
		0	0		
ElHa	Variant	15.8	15.4	0.9	
		21.2 (20.9)	19.4 (18.6)		
		25.8	20.9		

Cells were collected from monolayer cultures, suspended in Waymouth's medium containing 10% immunoprecipitation tested (IPT) foetal calf serum, and inoculated into replicate T-30 flasks (Falcon) at concentrations of 10^5 cells cm^{-2} of surface area (2.5×10^6 cells per flask). They settled into dense monolayers within 6–8 h at 37°C . Sendai virus, inactivated with β -propiolactone¹², was added to half of the flasks at a concentration of 1,000 to 5,000 haemagglutination units per flask. The flasks without virus served as controls. After overnight incubation, the medium was decanted, fresh medium containing 10% foetal calf serum was added, and the flasks were maintained with routine feeding until assay from three to eight days later. The virus-treated flasks contained 15–30% multi-nucleated cells while control flasks had 1–2%.

BCKA decarboxylase was measured by collecting the cells from replicate flasks with a brief exposure (1–2 min) to trypsin (0.04%) and versene (0.02%) in Puck's saline A, followed by scraping with a rubber policeman. Each suspension was diluted with foetal calf serum so that the final serum concentration was 20%, centrifuged at 800 r.p.m. for 5 min, and washed once with Hank's balanced salt solution. After a second centrifugation, the cell pellets were resuspended in 0.28 ml. of Krebs-Ringer phosphate buffer (pH 7.1–7.3) containing 1.75 mg ml^{-1} thiamine HCl and added to 21×70 mm homoeopathic vials which contained 0.5 μmol (0.2 μCi) of $[1-^{14}\text{C}]\text{-L-leucine}$. Less than 1% of the substrate was metabolized in the assay. Volatile radioactive contaminants were removed from the substrate before incubation as previously described^{1,7}. The vials were stoppered with serum vial caps carrying centre wells (Kontes, K882310 and K882320) containing 0.1 ml. of 1 N KOH, flushed with oxygen, and incubated at 35°C for 90 min with gentle swirling in a New Brunswick shaker water bath. The evolution of CO_2 is proportional to time for at least 120 min.

The reaction was ended by injecting 0.3 ml. of 10% TCA into the medium through the stopper, and the incubation was continued for an additional 60 min to complete the collection of dissolved CO_2 . The contents of the wells were transferred quantitatively to scintillation vials containing 15 ml. of scintillation cocktail¹. Reagent blanks normally liberated 20–40 c.p.m. of $^{14}\text{CO}_2$.

The TCA-precipitated cells were collected by adding 5 ml. of 5% TCA to the reaction vials and transferring the contents to centrifuge tubes. The precipitates were washed once with additional 5% TCA, once with distilled water and dissolved in 0.2 ml. of 1 N NaOH at 60°C for 2–4 h. The digests were added to scintillation cocktail to determine radioactivity.

BCKA decarboxylase activities in cultures fused with Sendai virus were compared to untreated replicate cultures (controls) prepared from the same cell suspension. Within an experiment the conditions of culture, the preparation of cells for assay and the measurements of BCKA decarboxylase activities were the same for the virus-treated and control cells.

* Ratio of $^{14}\text{CO}_2$ per 100 c.p.m. in TCA-insoluble material.

† Complementation ratio is the average BCKA decarboxylase activity of fused cells divided by that of control cells.

whether the cell strains were derived from normal subjects or from patients with classical or variant forms of MSUD (complementation ratio = 1). Similarly, if fusion was induced between fibroblasts derived from siblings with MSUD, who presumably have the same genetic lesion, there was no increase in enzyme activity (not shown). The two experiments carried out with GaSa (normal) in Table 1 show that the BCKA decarboxylase activity of a cell strain can vary from experiment to experiment. The method of assaying intact cells, involving complex handling of small numbers of cells which may differ in metabolic efficiency, probably introduces this variability. Within each experiment, however, we can obtain consistent measurements of enzyme activity in replicate flasks, thus allowing comparisons between virus-treated and untreated (control) flasks which have been handled similarly.

Once it was clear that the events of fusion, including the addition of virus, did not affect BCKA decarboxylase activity, fusion between MSUD strains were undertaken. BaKu has the clinical symptoms of classical MSUD, but when fibroblasts from this patient were fused with those of TeHe, NiRo, and ElHa, the virus-treated flasks consistently had BCKA decarboxylase activities at least three times greater than those of the same cell mixtures which were not treated with virus (Table 2). In each instance the enhanced activity was the result of increased evolution of radioactive CO_2 while the radioactive TCA-precipitated material remained constant. Complementation occurred regardless of whether the partners of BaKu had similar or different clinical manifestations of MSUD.

Table 2 Fusions between BaKu and other MSUD Fibroblast Strains

BaKu (Classical)	Fused with:	Expt.	Day of assay	BCKA decarboxylase activity		Complementation ratio
				Control cells Individual flasks (Average)	Fused cells Individual flasks (Average)	
TeHe (Classical)	1	4	10.8	38.8	(22.7)	6.3
			0 (3.6)	18.0		
			0	11.3		
		5	2.1	15.5	(21.5)	30.7
			0 (0.7)	31.7		
			0	17.3		
	2	3	2.3	31.2	(35.1)	7.5
			5.3 (4.7)	36.0		
			6.5	38.2		
		4	6.0	23.1	(32.0)	4.0
			4.0 (8.0)	38.2		
			14.0	34.6		
NiRo (Variant)	3	5	5.8	27.9	(41.2)	4.1
			6.7 (10.0)	46.6		
			17.5	49.0		
	4	4	41.7	138.5	(134.2)	3.6
			31.1 (37.8)	141.4		
			40.5	122.7		
ElHa (Variant)	5	3	7.5	32.4	(43.7)	4.7
			10.9 (9.3)	52.9		
			8.0	41.0		
	6*	6	9.1	37.8	(63.2)	3.1
			21.2 (20.6)	70.6		
			19.9	55.8		
	7	4	23.0	84.5	(75.7)	4.3
			14.2 (17.6)	85.6		
			15.7	56.9		
	6		11.6	61.0	(54.3)	4.9
			9.4 (11.1)	46.6		
			12.2	55.2		

Fibroblasts from two individuals were mixed in equal numbers and distributed among T-30 flasks. Half the flasks were treated with inactivated Sendai virus and half were controls. The BCKA decarboxylase activities were determined with leucine as substrate at the same time on different days after fusion.

Decarboxylase activity and complementation ratios are reported as described in Table 1.

* ElHa fibroblasts from exp. No. 6 when assayed alone had BCKA decarboxylase activity of thirty and thirty-one units.

Table 3 Fusions among MSUD Fibroblast Strains

Fusion	Expt.	Day of assay	BCKA decarboxylase activity		Complementation ratio
			Control cells Indi- vidual flasks	Fused cells Indi- vidual flasks (Aver- age)	
TeHe × ElHa (Classical × variant)	1	4	9.9	17.9	0.8
			13.5	4.8 (15.8)	
			36.8	24.6	
	2	7	49.6 50.3 58.6	42.8 44.8 60.1 (49.2)	0.9
NiRo × ElHa (Variant × variant)	3	4	76.5	29.5	0.4
			89.9	25.5 (34.3)	
			98.9	48.0	
	6		79.3	41.4	0.4
			88.3 83.7	31.3 32.3 (35.0)	

See legends of Tables 1 and 2.

The effectiveness of BaKu in complementing the three other mutants suggests that this patient's genetic lesion is distinct from the others, but it does not necessarily imply that the other three have the same genetic lesion. This aspect was investigated by fusions among these three (Table 3). BCKA decarboxylase activity did not change significantly when TeHe was fused with ElHa. Similar studies with NiRo and ElHa, however, produced a distinct depression of BCKA decarboxylase activity. This might suggest that the mutations of these two subjects differ such that their interaction lowers the effectiveness of the enzyme, perhaps by a mechanism similar to that of complementation but with a negative effect. Clinically, the possibility arises that the progeny of two such patients might be more enzyme deficient than either parent.

The genetic lesion in MSUD causes approximately parallel deficiencies in decarboxylase activity toward the three branched-chain amino-acids, suggesting that in normal individuals a single enzyme is responsible for decarboxylating all of them.

When leucine and valine were compared as substrates for the activity produced by complementation restoration of decarboxylase activity for leucine (complementation ratio 3.9) was much greater than for valine (ratio 1.5) in a typical experiment. Although this finding may indicate that there is a separate decarboxylase enzyme for each substrate, it might also be the case that the enzyme activity restored by complementation differs enough from the wild-type to metabolize the substrates selectively. Interallelic complementation in microbial systems and in eukaryotic cells often restores enzyme activity with kinetic properties different from the wild-type.

The enhanced BCKA decarboxylase activity found in virus-treated mixtures of cells from certain patients deficient in this enzyme activity strongly suggests that complementation has occurred in heterokaryons produced by cell fusion. Complementation appears to be selective, as it was observed with some pairs of cell strains but not with others. This finding indicates that MSUD fibroblast strains fall into at least two complementation groups which do not appear directly to be related to the clinical expression of the disease. An important finding in our experiments is that this technique can be used to study monolayer cultures which have been exposed to inactivated Sendai virus without further selection. The complementation that we observe must be very efficient, as we estimate that only about 18% of the virus-treated cells were actual heterokaryons, based on random fusion of the two cell types. Thus the level of BCKA decarboxylase activity may be close to the normal level in the few heterokaryotic cells which can express the enhancement.

It is not possible, on the basis of our observations, to distinguish between intergenic and interallelic complementation. The peptide composition of the BCKA decarboxylase and the

number of genetic loci which contribute to the enzyme complex are unknown. Complementation occurs in conditions of culture where nuclear fusion is unlikely. This strongly suggests that in our heterokaryons cytoplasmic interactions are responsible for complementation. BCKA decarboxylase seems to have a mitochondrial localization in liver⁹⁻¹¹ and to be part of a large multicomponent enzyme complex. To determine how complementation occurs requires a cell-free enzyme preparation, but we have been unable to recover significant BCKA decarboxylase activity from disrupted normal or mutant fibroblasts.

Complementation analysis provides a potentially important method for studying the genetic heterogeneity observed in many human biochemical disorders. For example, complementation can reveal functional differences between mutations of independent origin. Using these techniques it may be possible to construct complementation maps of various inborn errors and eventually to understand the molecular basis of the disease at the level of the deficient enzyme.

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LINDA B. LYONS*
R. P. COX
J. DANCIS

*Departments of Medicine,
Pharmacology and Paediatrics and the
Stella and Charles Guttman Laboratory
of Pharmacogenetics, New York University Medical Center,
New York, NY 10016*

Received January 22; revised March 9, 1973.

* Present address: Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, Md. 20014.

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Role of Inositol Cyclic Phosphate in Stimulated Tissues

THYROID-STIMULATING hormone markedly stimulates phosphatidylinositol turnover in sheep thyroid gland. The enzyme catalysing the degradation of phosphatidylinositol in animal tissues can split the phospholipid, with the forma-

tion of a diglyceride and a new cyclic ester, D-myoinositol-1:2-cyclic phosphate as well as D-myoinositol-1-phosphate^{2,3}. Increased phosphatidylinositol turnover has been described in a variety of conditions in which cells are stimulated to secrete or into other hyperactivity. The physiological possibilities of this degradation are obvious, and it was suggested^{2,3} that the catabolism of phosphatidylinositol and the release of inositol cyclic phosphate might be more directly relevant to the cell stimulation than to the biosynthesis involved in the turnover. Such an hypothesis is attractive, for an accumulation of phosphatidylinositol has never been demonstrated during stimulated conditions. In addition Ca^{2+} is an essential requirement for the formation of inositol cyclic phosphate as well as playing an important part in secretory processes⁴.

The demonstration of a specific phosphodiesterase in many mammalian tissues which hydrolyses only the D-enantiomorph of inositol cyclic phosphate, producing inositol-1-phosphate⁵, also suggested a defined function for the metabolite. We therefore tested whether a direct role for inositol cyclic phosphate could be demonstrated in the phenomena associated with increased phosphatidylinositol turnover of stimulated tissues. Michell and Lapetina^{6,7} have expressed similar speculative views concerning the possible role of inositol cyclic phosphate in the stimulated turnover of phosphatidylinositol.

The phosphodiesterase which attacks inositol cyclic phosphate is similar to the enzyme hydrolysing adenosine cyclic phosphate, having a requirement for Mg^{2+} and an alkaline pH optimum⁵. But we have found that the enzymes have distinct identities. Thus, the distribution of inositol-1:2-cyclic phosphate-2-phosphohydrolase is quite different from that of cyclic AMP phosphodiesterase in the rat kidney nephron. The former enzyme is confined to the brush borders of the pars convoluta in the outer kidney cortex, whereas the latter occurs chiefly in a soluble fraction obtained from the medulla and papilla. Furthermore, kidney inositol-1:2-cyclic phosphate-2-phosphohydrolase is insensitive to theophylline, a potent inhibitor of cyclic AMP phosphodiesterase, in the same tissue^{8,9}. We have also shown, in collaboration with Drs L. Birnbaumer and E. Whitmore (Department of Physiology, Northwestern University Medical School) that inositol cyclic phosphate does not act as a competitive inhibitor of cyclic AMP phosphodiesterase. These experiments were performed with a variety of rat tissues using a substrate concentration of $0.25 \mu\text{M}$ ^{32}P -labelled cyclic AMP (high affinity enzyme)¹⁰: even inositol cyclic phosphate at a concentration of 10^{-3} M produced no inhibition.

In additional experiments performed in collaboration with Dr L. Birnbaumer we found that inositol cyclic phosphate at a final concentration of 10^{-4} M had no effect on the adenylcyclase system of renal medullary membranes. This activity was determined at two [$\alpha^{32}\text{P}$] ATP concentrations (5 and $64 \mu\text{M}$) and in the absence and presence of arginine, vasopressin, prostaglandin E_1 , GTP and adenosine—agents which profoundly affect the activity of the cyclase.

In further experiments in collaboration with Dr J. F. Wilber (Department of Medicine, Northwestern University Medical School) we tested whether inositol cyclic phosphate had any effect on the elaboration of growth hormone (GH) and thyroid stimulating hormone (TSH) by hemisected rat anterior pituitaries. The pituitary shows an increased turnover of phosphatidylinositol in response to secretory stimulation¹¹. The excised rat hemipituitaries, after a stabilizing incubation, were incubated with and without inositol cyclic phosphate and the release of growth hormone and thyroid stimulating hormone determined by radio-immunoassay¹². No enhanced TSH or GH release was seen with pure Na inositol cyclic phosphate at concentrations up to 10 mM.

Increased phosphatidylinositol turnover is one of the primary events when lymphocytes are induced to transform

with phaeohaemagglutinin¹³ or concanavalin A (unpublished results of M. Smith and R. M. C. D.). In collaboration with Dr A. C. Allinson (National Institute for Medical Research, Mill Hill, London), however, we found no evidence that inositol cyclic phosphate could by itself transform sheep lymphocytes. Nor did concanavalin A increase the ^{32}P labelling from ^{32}P -inorganic phosphate of a fraction from lymphocytes containing inositol cyclic phosphate, even though the labelling of phosphatidylinositol was increased ten-fold in the first hour. In addition, in experiments with Dr G. E. Shambaugh III (Department of Medicine, Northwestern University Medical School) in which human lymphocytes were prelabelled by incubation with ^{14}C -inositol, we could not demonstrate a measurable release of labelled inositol cyclic phosphate within the lymphocytes when phaeohaemagglutinin was added to the incubation medium.

Finally, we prelabelled isolated pancreatic islets of the rat by incubation with both ^{32}P -phosphate and ^3H -inositol. On perfusion of such islets according to the technique of Lacy *et al.*¹⁴ an increase of glucose concentration from 50 to 300 mg/100 ml. almost immediately elicited a stimulated secretion of immunoreactive insulin. But no release of inositol cyclic phosphate or its immediate enzymatic decomposition product, inositol-1-phosphate, could be detected, either in the islets themselves or in the perfusion fluid. Stimulation of insulin release from rat pancreas slices by an elevated glucose concentration is again attended by an increased incorporation of precursor isotopes into phosphatidylinositol¹⁵.

In none of the systems which we have examined, and within the limitations of existing analytical techniques, can we find evidence that in a stimulatory situation there is an increased level or release of the metabolite inositol cyclic phosphate. Nor can we find any evidence that this metabolite can enhance hormone release, transform lymphocytes or affect the formation or breakdown of cyclic AMP. Of course, it is almost impossible to prove a negative physiological function for any individual metabolite, particularly when tissues contain a very active enzyme system for degrading this metabolite. So far, however, we have been unable to secure evidence that the enhanced turnover of phosphatidylinositol observed during many types of tissue stimulation can be ascribed to a need to produce inositol cyclic phosphate, and further speculation on this score without firm experimental data is useless.

N. FREINKEL

Endocrinology Center for Metabolism and Nutrition,
Northwestern University Medical School,
Chicago, Illinois

R. M. C. DAWSON

Biochemistry Department,
Institute of Animal Physiology,
Babraham, Cambridge

Received February 13; revised March 6, 1973.

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Oviposition Pheromone in Larval Mandibular Glands of *Ephestia kuehniella*

WHEN larvae of *Ephestia kuehniella* Zeller meet they deposit from their mandibular glands a pheromone that mediates a response to crowding¹. In this communication I show that ovipositing female moths of *E. kuehniella* respond to the same secretion in such a way that the number of eggs they lay is related to the amount of the larval pheromone (and so to the density of the population of larvae) at the oviposition site.

The experimental animals were kept at 25° C in a photoperiodic regime of 12 h light : 12 h dark, from the wandering stage of the last larval instar onwards. Males and females were kept in separate incubators. Each day at lights-off the moths that had emerged during the past 24 h were removed from the emergence jars. Twenty-four hours later the females were put with an equal or greater number of males of the same day-group; and 24 h after that the females (2-d-old mated females) were used in experiments. Although most of the females treated in this way mate², some of the females that laid few eggs contained unusually small spermatophores and some contained none. Females that laid fewer than ten eggs have therefore been omitted from the analysis.

Two-day-old mated females, placed singly in an inverted vial supported over a horizontal disk that rotated once in 24 h, laid most of their eggs on the disk just after the beginning of the dark period, sometimes showing a smaller peak of oviposition before lights-on (Fig. 1). The number of eggs decreased from a maximum on the first day after mating to a very few on the fourth. The experiments described here were arranged so that fresh mandibular gland material was presented to the 2-d-old mated female moths at lights-off, just before the first oviposition peak.

In the first experiment, 2-d-old mated females of *E. kuehniella* were put singly into plastic boxes (of internal dimensions 56×38×13 mm) in which wandering larvae of the same species had just been confined (singly or in groups of five, ten, twenty or fifty) for 1 h at 25° C. These larvae were removed from the boxes a few minutes before lights-off, when the moths were put in and allowed to oviposit for 24 h. Figure 2 shows that the number of eggs laid in each box is related to the number of larvae previously

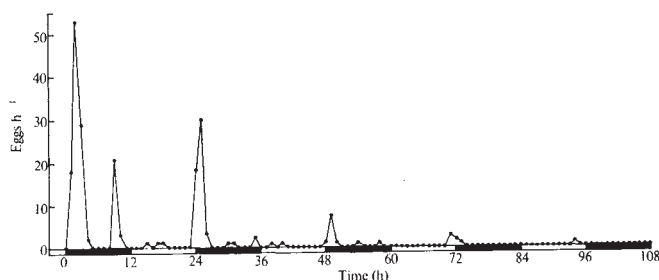


Fig. 1 Temporal pattern of oviposition for a 2-d-old mated female moth in a photoperiodic regime of 12 h light : 12 h dark (indicated on the abscissa).

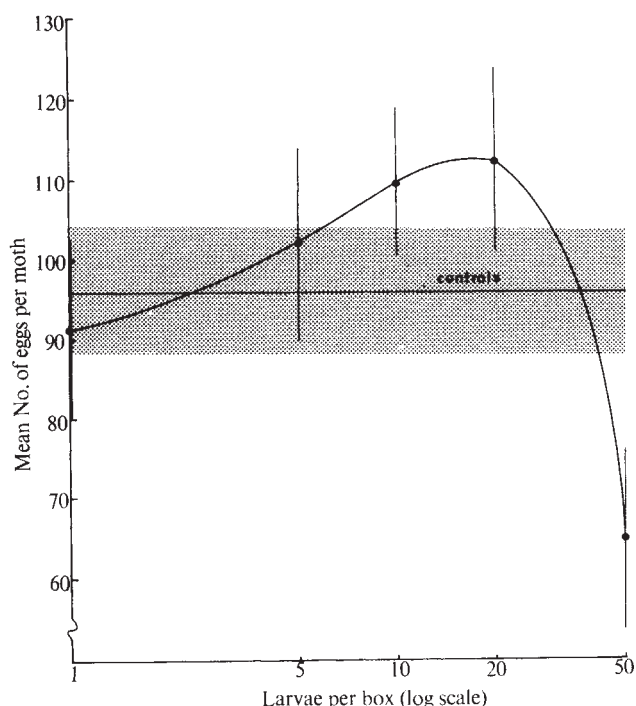


Fig. 2 Numbers of eggs laid in 24 h by 2-d-old mated female moths confined alone in standard boxes that had been previously inhabited for 1 h by single or crowded larvae. The horizontal line represents the mean number of eggs laid in control boxes that had not contained larvae. The vertical lines (and the extent of stippling for the controls) represent 1 s.e. each side of the mean.

crowded there; more eggs were laid in boxes that had contained about twenty larvae than in boxes that had contained more or fewer larvae. The second experiment showed that this response could be mediated by mandibular gland secretion deposited by the crowded larvae. Mandibular glands from wandering larvae were ground up in ether, and 50 μ l aliquots of various dilutions of the ether extract were put onto 16 mm diameter coverslips. Within 15 min of lights-off, 2-d-old mated female moths were put singly into standard boxes, each with a treated coverslip, and allowed to oviposit for 6–7 h. Figure 3 shows that more eggs were laid at about 0.05–0.5 pairs of glands per coverslip than were laid at higher or lower concentrations of mandibular gland extract. The concentration of the mandibular gland extract influenced not only the number of eggs laid during the first oviposition peak but also the spatial distribution of the eggs; the percentage of eggs laid on or under the coverslips was higher for those treated with 0.5 pairs of glands than for those treated with higher or lower concentrations of extract.

Ether extract of mandibular glands contains several components, but it is possible to obtain one of them almost pure by squeezing the oily fluid out of mandibular glands under water and drawing off the water (compound I of Mudd and Corbet³). The third experiment showed that the effect on oviposition of material prepared in this way was similar to that of the ether extract. Within 15 min of lights-off, twenty-one 2-d-old mated female moths were put singly into standard boxes, each of which contained a 16 mm diameter coverslip bearing the squeezed-out contents of one mandibular gland from a wandering larva. This oily droplet was dispersed over the coverslip by adding a 50- μ l aliquot of ether. In the first 6 h from lights-off these moths laid an average of 123 eggs each (s.e.=14). A parallel series of twenty moths with clean coverslips laid significantly fewer eggs (mean egg number=97; s.e.=9; $P<0.05$, one-tailed Mann-Whitney U test). Further, the proportion of

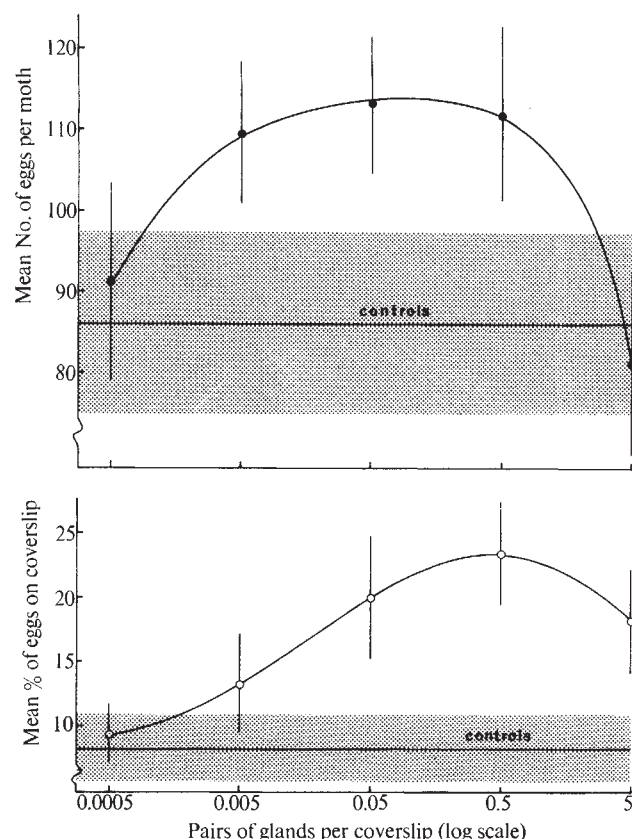


Fig. 3 Influence of concentration of ether extract of mandibular glands on (a) the number of eggs laid in 6–7 h by 2-d-old mated female moths; and (b) the percentage of those eggs that were laid on the coverslip bearing the extract. The horizontal lines represent the mean values for controls with untreated coverslips. The vertical lines (and the extent of stippling for the controls) represent 1 s.e. each side of the mean.

eggs laid in contact with the coverslip was significantly higher for the treated coverslips (mean=22%; s.e.=4%) than for the clean ones (mean=9%; s.e.=1%; $P<0.01$, one-tailed Mann-Whitney U test).

The response of larvae to larval mandibular gland secretion could contribute to the regulation of population density, affecting the dispersion of larvae, the duration of larval development and the size (and therefore the fecundity) of moths crowded as larvae¹. The experiments described here show that the moths, too, respond to the secretion in a way that could contribute to the regulation of population density. The larval and the imaginal responses show similar patterns of change with the concentration of mandibular gland extract. The duration of the last larval instar is least at an intermediate concentration of gland material (corresponding to the material deposited by five larvae in a standard box in 1 h) and longer at lower or higher concentrations; and the number of eggs laid by moths is greatest at an intermediate concentration (corresponding to the material deposited by about twenty larvae in a standard box in 1 h) and less at higher or lower concentrations. The pattern of the moths' response may enable them to select an oviposition site that is capable of supporting larval growth, but is not over-populated.

Among pheromones with potential for the control of insect pests those affecting the time or place of oviposition are especially worth attention as they can be used to prevent or reduce infestation by repelling gravid females or by luring them into traps. Compound I, which is probably at least partly responsible for the effects on oviposition described here, has been identified in the larval mandibular glands of three other phycitid pests of stored products,

Ephestia cautella (Walk.), *E. elutella* (Hübner) and *Plodia interpunctella* (Hübner) as well as *E. kuehniella*³, and work is in progress to establish its structure.

SARAH A. CORBET

Westfield College,
London NW3 7ST

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Ventilation of Toad Lungs in the Absence of the Buccopharyngeal Pump

T. H. HUXLEY¹ stated "Pulmonated Vertebrata, which have the thoracic skeleton incomplete (as the Amphibia) breathe by distending their pharyngeal cavity with air; and then, the mouth and nostrils being shut, pumping it, by the elevation of the hyoidean apparatus and floor of the pharynx, into the lungs. A frog therefore cannot breathe properly if its mouth is kept wide open". This account of amphibian respiration and the buccopharyngeal "pump" has been perpetuated in biological texts (for instance, refs. 2–7) but, as our observations on the toad *Bufo marinus* indicate, may not be completely correct.

The mouths of the toads (*Bufo marinus*, 150 to 350 g) were held open by thrusting a piece of plastic tubing between the jaws. These toads cannot inflate their lungs to the balloon-like proportions often seen, the buccopharyngeal pump being necessary for this and also undoubtedly for normal respiration. These toads, however, rapidly (20–30 per min; mean 26 for 7 toads) oscillated their dorsal thoracic wall. Oxygen consumption was greatly in excess of cutaneous oxygen uptake^{8–10}, thus confirming that ventilation of the lungs did occur.

Table 1 Oxygen Consumption of *Bufo marinus* (at 22° C). Effects of Preventing Ventilating Action of the Buccopharyngeal Pump

Wt. of toad (g)	Oxygen consumption ml. kg ⁻¹ h ⁻¹		
	Normal "resting"	Buccopharyngeal "pump" blocked (tube in mouth)	Injected with neuromuscular blocker (gallamine)
145	41	84	—
351	42	113	42
311	44	150	35
265	57	252	55
261	43	166	55
240	63	106	46
237	33	148	54
227	64	179	46
Mean ± s.e.	48 ± 4.0	150 ± 18.5	48 ± 2.9

The rate of resting oxygen consumption (measured with a paramagnetic oxygen analyser) is shown in Table 1. When the tube was placed between the jaws oxygen consumption did not decline, as may be expected if ventilation of the lungs ceased, but rose considerably. This continued for at least 2 h. The increased metabolism may reflect excitement as well as increased muscular activity of breathing. The toads did not appear distressed and the breathing movements were not gasping ones. To prevent movements of the thoracic muscles and thus stop ventilation of the lungs, we injected a neuromuscular blocking drug (gallamine triethiodide, 40 mg per 150–350 g toad). After 60 to 90 min the thoracic breathing movements usually ceased. They retained the eye blinking reflex, however, and buccopharyngeal movements could still occur so that the mouth-tube was left in place. In these toads, oxygen consumption declined to the original "resting" value. This presumably reflects enhanced cutaneous oxygen uptake.

There has been considerable speculation regarding the possible respiratory mechanism present in early amphibians. Cox¹¹ considers that the ancestral Lissamphibian relied solely on hyoid (positive-pressure pumping) ventilation supplemented by cutaneous gas exchange. Gans¹², on the other hand, has suggested that such amphibians must have utilized aspiratory (negative-pressure type) ventilation and that the hyoid ventilation in the frog is a "red herring". The present observations indicate that negative-pressure type ventilation may occur even among contemporary amphibians despite the lack of a diaphragm and rib cage. Thus the present experimental observations pose two fundamentally interesting questions. First, how essential is the rib cage and diaphragm for negative-pressure type "ventilation"? Second, need we assume that the earliest terrestrial vertebrates possessed a buccopharyngeal pump?

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P. J. BENTLEY
J. W. SHIELD*

*Departments of Pharmacology and Ophthalmology,
Mount Sinai School of Medicine of the
City University of New York,
New York, NY 10029*

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* Present address: Department of Zoology, University of Western Australia, Nedlands, Western Australia.

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Absence of Y-Body in the Cervical Mucus of Pregnant Women

SHETTLER¹ reported the possible use of the fluorescent Y-chromatin test² for prenatal sex diagnosis in smears of maternal cervical mucus. This technique, if it works, could be very valuable, particularly during the first trimester of pregnancy when amniocentesis is impracticable. We have tested the feasibility of the technique in sixteen pregnant women who were admitted for legal abortion in the first trimester, that is, from the seventh to twelfth week of gestation after the first day of the last menstrual period.

Smears obtained from mid-cervical mucus with a cotton swab were fixed in 3:1 methanol-acetic acid for 1-2 min, air-dried and stained with quinacrine mustard (50 $\mu\text{g ml}^{-1}$ in MacIlvaine citric acid-phosphate buffer, pH 4.5) for 10 min. They were washed in running tap water, rinsed three times and mounted in the same buffer. In each sample 100-800 interphase nuclei were scanned for the Y-body, using a fluorescence microscope. Conceptuses were then taken out by curettage, and fragments of the embryo and chorionic villi separated, washed in saline, minced with scissors, and smeared on slides. The slides were examined for the Y-body as described above. The X-chromatin of

both villi and embryonic tissues was examined according to the acetic orcein squash method, on the basis of 100 nuclei in each sample. In addition, a portion of the embryonic tissue was cultured *in vitro* for direct analysis of sex chromosomes.

Twelve of the sixteen embryonic cell cultures showed a 46,XY karyotype and the remaining four a 46,XX karyotype. The X-chromatin findings in both chorionic villi and embryonic tissues conformed with the results of the karyotype analysis. In spite of the relatively high proportion of male conceptuses involved, we failed to detect typical Y-body in any of the sixteen samples of cervical smears, which were scanned blind on the basis of a total of 4,000 well-fixed nuclei. In striking contrast, the smears of chorionic villi and embryonic cells from the twelve chromosomally verified male specimens, showed a very high incidence of the Y-body; it was present in 70% of villi smears and 92% of embryo smears. Smears from the remaining four cases did not show Y-body in the villi, embryonic cells and the cervical mucus. Some cells, however, had a less prominent fluorescent spot, probably of X-chromatin origin. This atypical body was also found in a few cervical cells of mothers of male fetuses, but was not regarded as the Y-body because of the rather indistinct morphology and the dispersed and weaker fluorescence.

In three of the sixteen women the uterine cavity was carefully perfused with 10 ml. of a sterilized balanced salt solution, and the resultant cellular component collected by centrifugation and examined for the Y-body. No Y-body was detected in these samples, though the embryos were later confirmed to be Y-positive. In one case, however, several tiny tissue fragments which appeared as clumps of cells on the slide were chorionic cells and Y-positive. Non-clumping free cells in the same slide showed no trace of the Y-body. It can be assumed that chorionic tissues were mechanically disrupted by the perfusion procedure.

The possibility of natural migration or shedding of chorionic cells into the cervical mucus early in embryonic development is, therefore, very unlikely, and clinical examination of cervical mucus in order to predict the sex of the embryo is unwarranted.

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K. TSUII
M. SASAKI

*Chromosome Research Unit,
Faculty of Science,
Hokkaido University,
Sapporo*

Received January 22, 1973.

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Role of Erythrogenin from Liver and Spleen in Erythropoietin Production in the Anephric Rat

It is well established that the kidney is the main site of the production and/or activation of erythropoietin (Ep)^{1,2}. Gordon *et al.*³ have presented evidence indicating that under both normal and hypoxic conditions the production of circulating Ep involves the interaction of a renal erythropoietic factor (also termed erythrogenin) with a serum protein component. Erythrogenin can be extracted from the light mitochondrial fraction prepared from homogenates of mammalian kidneys.

Extrarenal mechanisms of Ep production, however, seem to function in both humans⁴ and rodents⁵. Recent experiments

indicate that, although the capacity of anephric rodents to produce Ep in response to mild hypoxia (0.45 atm air) is gradually abolished within 12–24 h after nephrectomy^{6,7}, a sustained Ep production can be elicited in these animals by severe hypoxic stimuli (0.35–0.37 atm air)⁸. Intravenous administration of erythropoietin fully restores the ability of 24-h anephric rats to initiate Ep production when subjected to mild hypoxia⁶ and the liver has recently been implicated as a possible source of Ep⁹. The present studies were undertaken in an attempt to verify the role of both liver and spleen as possible sources of erythropoietin in extrarenal Ep production.

In all experiments, female mice of the CF1 strain weighing 20–25 g or male rats of the Long-Evans strain weighing 150–200 g were used. The animals were maintained on a diet of pellets and tap water *ad libitum*. Levels of Ep in test materials were assessed in ex-hypoxic polycythaemic mice according to a previously reported technique¹⁰. Results are expressed in terms of either % ⁵⁹Fe incorporation into red blood cells per 48 h or equivalent units of the International Reference Preparation (IRP) of Ep.

Sham operations, splenectomy or subtotal hepatectomy were performed 12 h prior to bilateral nephrectomy. One group of sham-operated animals did not have their kidneys removed. The rats were exposed to a bout of severe hypoxia (0.37 atm

air for 6 h) 13 h after the initial operation (1 h after nephrectomy). Sham-operated or nephrectomized rats maintained at room pressure were used as controls. Each group consisted of at least three animals. All operations were performed under light ether anaesthesia. Subtotal hepatectomy involved removal of both the middle and left lobes and two-thirds of the right lobe. The caudate lobe was not removed. Bilateral nephrectomy was performed by way of the ventral approach. Blood was collected by cardiac puncture under light ether anaesthesia immediately after the end of the hypoxic period. The plasma pooled from each group was dialysed against physiological saline for 24 h at 4° C and stored at –20° C until assayed.

Sham-operated or nephrectomized rats were subjected to hypoxia (0.37 atm air for 6 h) 1 h after the operation. The rats were totally exsanguinated immediately after the hypoxic period. Control animals, subjected to either sham operation or nephrectomy, were maintained at room pressure. The light mitochondrial extract (LME) from kidney, liver or spleen was prepared by a modification of the method reported by Cantor *et al.*¹¹ for renal LME (erythropoietin).

Serum obtained from normal male rats was dialysed against 100 volumes of 0.005 M Na₂EDTA for 24 h at 4° C. The material was redialysed against deionized water (100 volumes) for 24 h and the dialysate, normal rat serum (NRS), frozen at

Table 1 Ep Activity in the Plasma of Rats Subjected Sequentially to Sham Operation, Splenectomy or Subtotal Hepatectomy; Bilateral Nephrectomy; and Hypoxia (0.37 atm Air for 6 h)

Group No.	Treatment of donor rats	Amount of plasma injected (ml per assay mouse)	Mean % RBC- ⁵⁹ Fe incorporation ± s.e.m. in recipient assay mice	Equivalent IU Ep ml ⁻¹
1	Sham operation + hypoxia	0.1	8.08 ± 1.37	2.0
2	Sham operation + nephrectomy + hypoxia	1.0	11.67 ± 0.48	0.52
3	Splenectomy + nephrectomy + hypoxia	1.0	11.21 ± 0.85	0.46
4	Subtotal hepatectomy + nephrectomy + hypoxia	1.0	2.71 ± 0.29*	0.05
5	Sham operation + room pressure	1.0	0.81 ± 0.09	ND
6	Sham operation + nephrectomy + hypoxia	1.0	0.70 ± 0.05	ND
Standards (injected in assay mice)				
	Saline		0.51 ± 0.07	
	0.05 IU Ep		2.68 ± 0.12	
	0.20 IU Ep		8.10 ± 0.63	

**P* < 0.01 when compared with groups Nos. 2 and 3.
A minimum of three rats and six assay mice per group.
Assay in ex-hypoxic polycythaemic mice.

Table 2 Erythropoietic Activity of Light Mitochondrial Extracts (LME), incubated with either Saline or Normal Rat Serum (NRS), prepared from Kidney of Sham-operated Rats or Liver and Spleen of Nephrectomized Animals

Group No.	Treatment of donor rats	Incubation procedure of test material	Mean % RBC ⁵⁹ Fe incorporation ± s.e.m. in recipient assay mice
1	Sham operation + hypoxia	Kidney LME + saline	1.08 ± 0.04
2	Sham operation + hypoxia	Kidney LME + NRS	4.32 ± 0.27
3	Sham operation + room	Kidney LME + saline	0.74 ± 0.16
4	Sham operation + room	Kidney LME + NRS	1.53 ± 0.19
5	Nephrectomy + hypoxia	Liver LME + saline	0.88 ± 0.06
6	Nephrectomy + hypoxia	Liver LME + NRS	3.19 ± 0.22
7	Nephrectomy + room	Liver LME + saline	0.77 ± 0.08
8	Nephrectomy + room	Liver LME + NRS	1.36 ± 0.12
9	Nephrectomy + hypoxia	Spleen LME + saline	1.03 ± 0.10
10	Nephrectomy + hypoxia	Spleen LME + NRS	3.44 ± 0.51
11	Nephrectomy + room	Spleen LME + saline	0.50 ± 0.17
12	Nephrectomy + room	Spleen LME + NRS	1.14 ± 0.05
13	None	NRS + saline	0.71 ± 0.08
Standards (injected in assay mice)			
	Saline		0.62 ± 0.13
	0.05 IU Ep		2.09 ± 0.11
	0.20 IU Ep		5.18 ± 0.79

A minimum of three donor rats and six assay mice per group. Each mouse received a total of 2 ml of the incubation mixtures. The rats were either exposed to hypoxia (0.37 atm air for 6 h) or maintained at room pressure. Assay in ex-hypoxic polycythaemic mice.

–20° C until used. Both liver, spleen and kidney LME were incubated with equal volumes of NRS for 45 min at 37° C in a water bath incubator with constant shaking. In control vessels, LME and NRS were incubated with equal volumes of physiological saline under the same conditions. All incubation mixtures were stored frozen at –20° C until assayed.

Table 1 shows that significant levels of Ep are detected in the plasma of nephrectomized rats subjected to severe hypoxia. This activity appears to be approximately 25–30% of that exhibited by sham-operated controls. It has been shown previously that in anephric rats weighing approximately 250 g the Ep response to hypoxia is significantly lower (10–15% that of sham-operated controls)^{6,7}. No Ep activity is detected in the plasma of nephrectomized animals maintained under room pressure conditions. Although preliminary splenectomy does not modify Ep levels in the plasma of anephric rats exposed to hypoxia, subtotal hepatectomy abolishes extrarenal Ep production almost completely.

As shown in Table 2, the LME of both liver and spleen obtained from nephrectomized rats subjected to hypoxia, although by itself erythropoietically inactive, exerts a significant stimulatory effect in assay mice when incubated with NRS. A similar Ep-generating activity was detected in the LME (erythrocytogenin) extracted from the kidneys of sham-operated rats subjected to an equivalent hypoxic period. It is assumed that both the liver and spleen of nephrectomized, hypoxic rats show a significant level of erythrocytogenin activity although no Ep-generating activity was observed in the LME extracts of both organs obtained from anephric animals maintained under room pressure conditions. Thus, the correlation between the levels of Ep in the plasma and erythrocytogenin in the liver and spleen suggests that extrarenal Ep production is at least partially mediated by hepatic and splenic erythrocytogenin.

Although comparable erythrocytogenin activity per g of wet tissue was obtained from both liver and spleen, subtotal hepatectomy results in almost complete abolition of extrarenal Ep production, while splenectomy does not exert a significant effect. The prominent role of the liver in this phenomenon may be because of the larger weight of this organ as compared to the spleen. Gordon *et al.*¹² have presented evidence indicating that, in a patient with hepatoma associated with erythrocytosis, extracts obtained from the hepatic neoplasm served as a component for the *in vitro* generation of Ep by renal erythrocytogenin. Therefore, an additional hypothesis to explain the present observation assumes that the liver produces factor(s) other than erythrocytogenin necessary for Ep production.

The present studies indicate that Ep production in anephric rats subjected to severe hypoxia is mediated by extrarenal erythrocytogenin and that the liver seems to be the main functional source of extrarenal Ep production.

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C. PESCHLE
A. D'AVANZO
I. A. RAPPAPORT
S. RUSSOLILLO
G. MARONE
M. CONDORELLI

*Institute of Medical Pathology,
II Faculty of Medicine and Surgery,
University of Naples,
Naples*

Received February 26, 1973.

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Body Weight as a Criterion in Judging Bone Mineral Adequacy

APART from the extreme criterion of bone fracture, there is no definitive measurement of bone adequacy in terms of strength in relation to function. Comparison of bone mineral is usually made on the basis of age¹ or sex² without regard to the size or the physical status of the individual. This is in spite of Galileo's observation that the weight of a body is a function of the cube of its diameter whereas the strength of a bone is a function of the square of its diameter³. Galileo also conceived that a constant sized bone would support more weight if it were made of a stronger material, but did not seem to be aware that bones are subject to change in density. This is rather surprising as he discussed at some length the density of various substances. We now know that bone density varies and that the strength of both trabecular⁴ and cortical bone⁵ increases with density.

Integration of these observations provides an explanation of how bones of animals of various species, age, sex and position in the body maintain functional adequacy. It seems that not only would a heavier animal have proportionally larger bones, but a given sized bone could increase in density in response to increased body weight.

The experiment reported here was designed to test the effect on bone density of changing the weight borne by the bone, by applying weights to the lumbar region of sheep. Seventeen castrated male sheep were divided into two groups, and after pretreatment for 6 weeks, saddles made from hessian, with calico lined pockets, were placed over the loins of both groups for 7 weeks. Nine Merino wethers each carried saddles with 10 kg (which approximates full term twin foetal weight) of cast iron in the pockets and the control group of eight wethers carried wood shavings of comparable bulk in their saddles. The sheep were housed randomly in two enclosed stalls each of 12 m² floor area, and fed freely on lucerne and wheaten chaff, with cotton seed meal added at the rate of 30 g per sheep per day. The control sheep made an average liveweight gain of 3.68 kg during the experiment and weighted sheep 2.27 kg. Observations were made weekly, when total weight, γ -ray absorption of the tibial tarsal bone and the leg thickness (medio-lateral) over the tibial tarsal bone were recorded.

Estimation of γ -absorption was made by passing a collimated beam of radiation from ²⁴¹Am through the tibial tarsal bone by a self-locating cast placed on a sheep's tarsus, and the emergent radiation was read on γ -detecting equipment (Nuclear Enterprises Ltd scintillation counter DMI-1 and a variable energy level detecting spectrometer NE 8433) (our unpublished results). The thickness of the leg (bone and overlying soft tissue) through the tibial tarsal region was measured using dial-faced callipers. The absorption was calculated as

$$\mu t = 2.303 \log_{10} (I_0/I_t)$$

where μ is the absorption coefficient of the principal wavelength of ²⁴¹Am; t the thickness of leg across the tibial tarsal bone (cm), and I_0 the intensity of radiation through air and I_t the intensity of radiation through t .

Figure 1 shows the mean values of γ -absorption (μt) for the weighted and control sheep in relation to time. There was little

Table 1 Comparison of Mean Changes in Absorption of Radiation (μ , μt and μt^2) between Periods in Nine Weighted (W) and Eight Control (C) Sheep

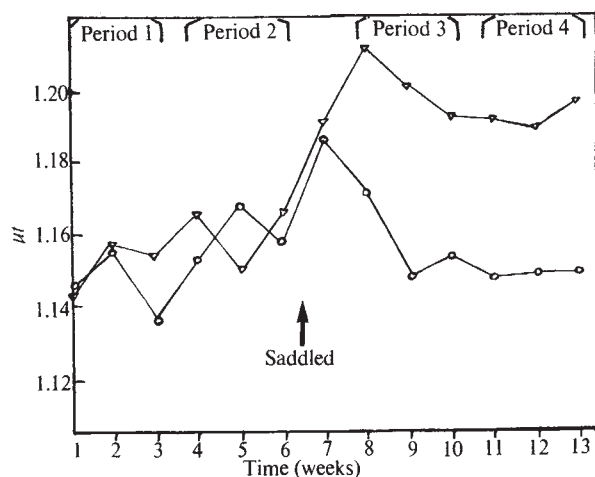
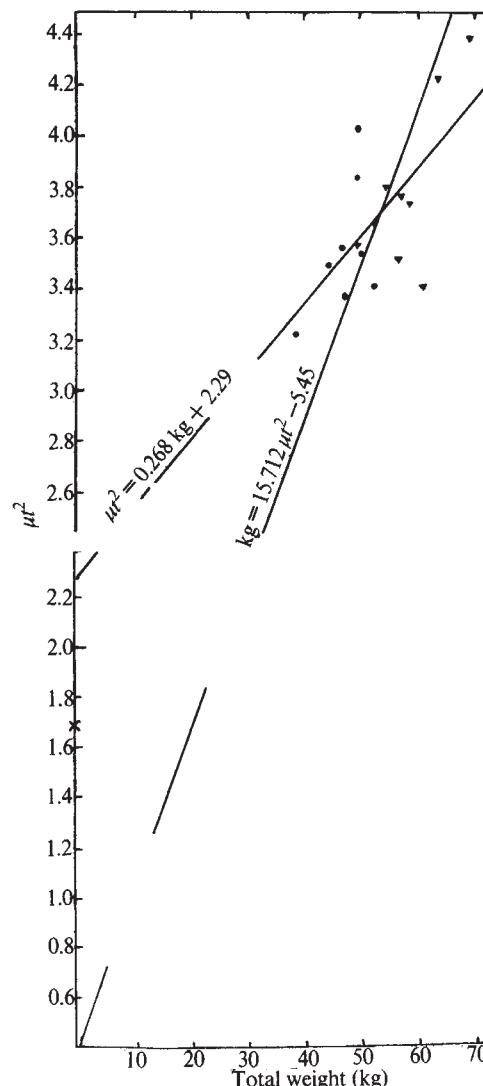
Comparison (periods)	$\Delta \mu \cdot 10^2$		$\Delta \mu t \cdot 10^2$		$\Delta \mu t^2 \cdot 10^2$	
	W	C	W	C	W	C
2-1	1.06	1.16	0.94	1.38	-5.54	-2.64
	$t=0.16$		$t=0.26$		$t=0.57$	
3-1	1.91	0.55	5.00	1.20	11.77	1.19
	$t=2.28^*$		$t=2.41^*$		$t=2.07$	
4-1	1.61	0.33	4.10	0.28	8.96	-1.80
	$t=2.92^*$		$t=4.55^\dagger$		$t=2.17^*$	
3-2	0.85	-0.62	4.10	-0.18	17.31	3.88
	$t=2.45^*$		$t=2.44^*$		$t=2.50^*$	
4-2	0.56	-0.63	3.18	-1.11	14.50	-0.84
	$t=3.05^\dagger$		$t=3.31^\dagger$		$t=3.20^\dagger$	

Each period represents the average of the mean of three successive weekly readings on each sheep. No sheep carried weights during periods 1 and 2; in periods 3 and 4 the weighted sheep carried 10 kg slung across their loins.

*, $P < 0.05$; † , $P < 0.01$; ‡ , $P < 0.001$; $df = 15$.

change during pretreatment but there was a rapid increase in γ -absorption in the weighted group to a maximum within 2 weeks of applying the extra weight. Because of large individual differences among the sheep, no significant differences between control and weighted groups could be detected by Student's t test at any time. Sheep in the treatment group apparently resented the weighted saddles and ran about excitedly when not physically restrained, while control animals showed evidence of a small increase in bone density immediately after the saddle was applied, possibly as a result of change in exercise pattern. More uniform readings were obtained in the later weeks when the animals became more placid, so tension on bone as a result of exercise could be important in influencing bone density measurements.

To test if the performance of the animals changed significantly as a result of the different treatments, the data were grouped into four periods of 3 weeks each as shown in Fig. 1. Periods 1 and 2 represented weeks 1-3 and 4-6 respectively (before the weights were applied), and periods 3 and 4 represented weeks 8-10 and 11-13 respectively. Week 7, being a transition week, was omitted from the grouping. The mean value for each animal within each period was obtained, and the changes for each animal between periods calculated. These mean values for changes in μt for control and weighted groups are shown in Table 1 together with the significance of differences between these means. No difference between the mean changes in the weighted and control groups between periods 1 and 2 was detected, but the changes from periods 1 and 2 to 3 and 4 are

**Fig. 1** The γ -ray absorption (μ) of eight control (○) and nine weighted (▽) sheep carrying 10 kg from week 6.**Fig. 2** Association between bone mineral (μt^2) of sheep tibial tarsal bones and the total weight of sheep (including saddles) after carrying saddles for 7 weeks. The μt^2 for tendon (X) is shown. ●, Control; ▼, weighted sheep, $r = 0.65$.

significantly greater in the weighted group than in the control group. A very highly significant difference between the change in γ -absorption of weighted and control groups occurred between periods 1 and 4.

Changes in leg thickness could account for changes in γ -absorption, but this is unlikely as the derived absorption coefficients (μ), which are independent of t , show differences of similar significance. Moreover, no significant changes in t were detected throughout the experiment. Unpublished work in this laboratory shows that there is a linear relationship between μ and the specific gravity of whole tibial tarsal bones of sheep ($r = 0.84$, $P < 0.001$). It seems then that the changes in γ -absorption are a result of changes in specific gravity of the bone rather than changes in leg size.

In order to estimate the total mineral in the leg, μ was multiplied by t^2 , that is, a density measurement times an estimated area across the leg, and designated μt^2 . These results are also shown in Table 1. The correlation between μt^2 and total weight at the thirteenth week is illustrated in Fig. 2 ($r = 0.65$, $P < 0.01$). Both regression lines are shown together with a point X on the y axis which is the μ for tendon (unmineralized collagen) multiplied by the square of the average leg thickness; this lies between the two regression lines.

Clearly, an increase in body weight produces a rapid increase in bone density, and the correlation between body weight and bone density indicates that the forces applied to a bone, whether

they be in the form of weight or tendon pull, determine the density of bone in individual animals in a relatively uniform manner. If this relationship holds for larger numbers of animals and is consistent across species, body weight must be regarded as an essential variable when judging bone adequacy. It should find application in assessing bone dystrophy and its treatment, bone mineral reserves in various physiological states such as pregnancy and lactation, and the adequacy of bone response during an exercise programme.

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N. J. SIEMON
E. W. MOODIE

Department of Veterinary Clinical Studies,
University of Queensland,
St Lucia, Brisbane

Received March 20, 1973.

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Variation in Trace Metal Concentrations along Single Hairs as Measured by Proton-induced X-ray Emission Photometry

TRACE elements in human and animal hair have been studied using spectrophotometric, atomic absorption, emission spectrographic, neutron activation, and spark source mass spectrometric methods. We report here measurements of trace elements in human hair using the technique of proton induced X-ray production.

Twenty-seven elements have been identified in human hair¹ in concentrations ranging from 0.1 to 100 μg^{-1} . Early measurements indicated a wide range of concentrations of almost all trace elements for different individuals but in certain cases correlations with sex, age, and colour of hair were reported²⁻⁶. It has been assumed that hair may be a good tool for studying the storage of nutrients by the body as it accumulates over a period of time and is relatively

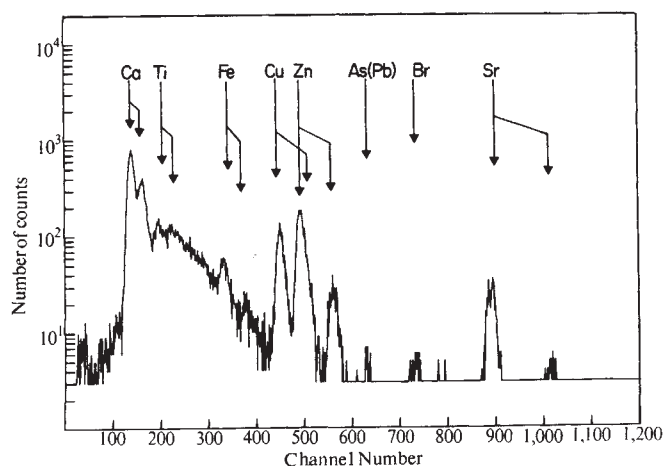


Fig. 1 X-ray spectrum obtained by exposing a single hair sample to a 3 MeV proton beam.

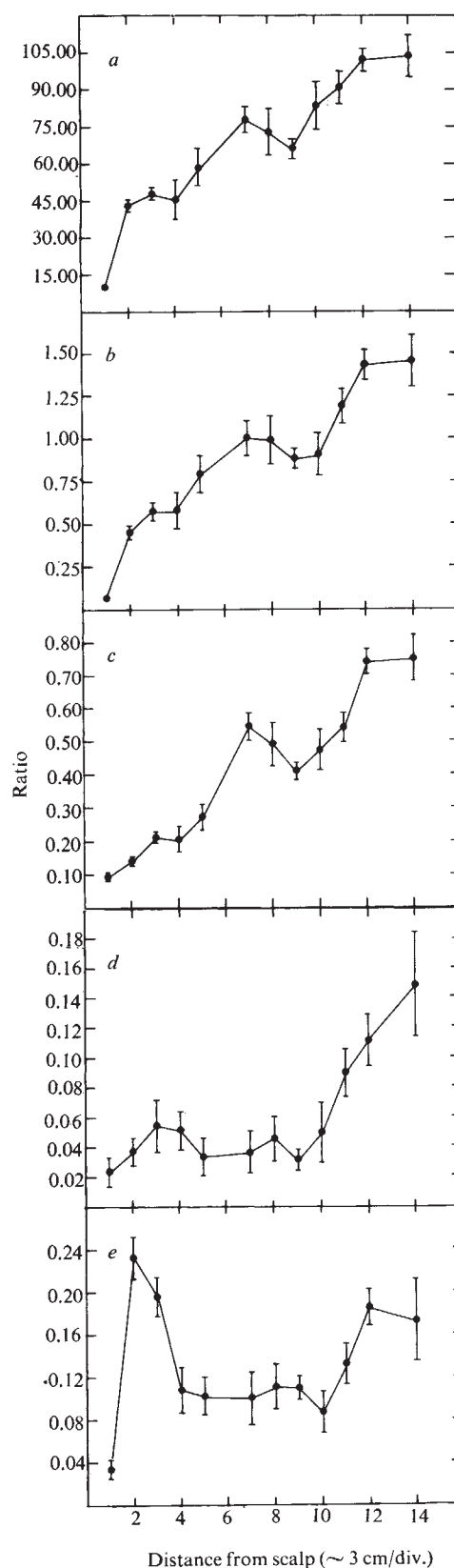


Fig. 2 Ratios of the element concentrations Ca/Zn (a), Sr/Zn (b), Cu/Zn (c), Br/Zn (d) and Fe/Zn (e), as a function of distance along the hair.

inert metabolically. Efforts to relate trace element content of hair to diseases which are known to influence trace elements in other parts of the body were not successful⁷⁻¹¹. Several research groups¹²⁻¹⁷ studied the level in hair of trace elements which could possibly be hazardous to health.

In the measurements reported here a single hair from a female head has been washed in triply distilled water, and cut into fourteen pieces approximately 3 cm in length. Each sample was then fixed to an aluminium frame. Each target was exposed to the beam of 3 MeV protons from the Rice University tandem Van de Graaff accelerator. Detector efficiency for elements lighter than Ca and the low energy bremsstrahlung background was artificially reduced by introducing a polystyrene absorber in front of the detector. The resulting X-rays were detected by a Si(Li) detector and amplified, then processed and stripped for peak intensity by an IBM-1800 computer. Figure 1 shows the X-ray spectrum obtained for one of the samples. Peaks associated with Ca, Ti, Fe, Cu, Zn, Pb, Br, and Sr K_{α} and K_{β} X-ray lines are present in all spectra.

The ratios of elements in the hair were normalized to ratios obtained from targets with known element concentrations. Figure 2 shows the ratios Ca/Zn, Cu/Zn, Sr/Zn, Fe/Zn, and Br/Zn as a function of distance along the hair from the scalp. The Ca/Zn, Cu/Zn, Sr/Zn ratios are found to increase monotonically with length along the hair except for a small dip in the 9–10 region. The Br/Zn and Fe/Zn ratios show a more complex structure, but also display a dip in 9–10 region. The Fe/Zn ratio in particular, undergoes very strong fluctuation near the base of the hair.

Two additional measurements were performed with beam-resistant and multihair targets using hair from the same female head. All three measurements were consistent. In addition, the shampoo which had been used in regular washing of the hair was found to contain no significant concentrations of metals heavier than Ca.

These results show that the concentrations of trace elements undergo large fluctuations in close proximity to the scalp. The relative concentrations of Ca, Sr, Cu, Fe, Br, and Zn change significantly with distance along the hair. The source of these variations may be associated with the environmental and biomedical history of the hair and its owner.

These facts must be considered when evaluating previous measurements of trace elements in hair. It is possible that some reported inconsistencies are due to the variation of element concentrations along single hairs and, therefore, future studies must pay attention to this problem.

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V. VALKOVIĆ
D. MILJANIĆ
R. M. WHEELER
R. B. LIEBERT
T. ZABEL
G. C. PHILLIPS

T. W. Bonner Nuclear Laboratories,
Rice University,
Houston, Texas 77001

Received January 15; revised March 5, 1973.

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Chemical Exploration of the Microhabitat by Electron Probe Microanalysis of Decomposer Organisms

INCREASING emphasis on the role of microorganisms as nutrient concentrators in ecosystems calls for the accurate, reliable chemical analysis of these cells within their microhabitats. The inability to perform such analyses has been a principal limitation of existing macrochemical techniques. Witkamp¹, Stark², and Todd and Cromack³ have demonstrated that terrestrial microflora can concentrate calcium, potassium and magnesium to a point that each becomes a major nutrient pool. Microarthropods—important grazers of microflora—link the terrestrial detritus-based food chain to other chains. This paper outlines a technique which enables the chemical composition of the microfloral and faunal biomass to be analysed without its destruction or separation from the detrital matrix. With the exception of laboratory bacterial cultures, all samples were collected from the US Forest Service Coweeta Hydrologic Laboratory field site, North Carolina.

Scanning electron microscopy is currently being used to provide "visual" observations of microorganisms in terrestrial⁴⁻⁶ as well as estuarine ecosystems⁷. Electron probe microanalysis utilizes a similar, focused high-energy beam of electrons to excite the constituent atoms of a sample to emit characteristic X-radiation. This radiation can be analysed and displayed as a scanned image of elemental concentration in the specimen or, in the static mode, quantitative spot analyses can be made by comparison with suitable standard samples. (With our instrument both were possible in the same run.)

The principles of electron microprobe analysis are discussed in detail by Birks⁸. He states that 1 μ g samples can be analysed with one part per billion resolution for the heavier elements (atomic number greater than 12). The precision and resolution, however, depend greatly on the nature of the sample and its preparation. Impregnation with plastic or metal, polishing to a smooth, flat surface, and vacuum-coating with carbon have usually been considered necessary. In this study, however, useful quantitative as well as qualitative data were obtained using the less disruptive preparation techniques outlined by Todd *et al.*⁷ for observing biological samples in the scanning electron microscope.

For examination and analysis, our specimens were affixed by double adhesive cellulose tape or silver paint to the surface of a specimen stub. The air-dried specimens were then coated (200–400 Å) with a gold-palladium alloy by vacuum evaporation. Secondary electron images were observed using a Cambridge Stereoscan Electron Microscope (Mark II A, Cambridge Instruments Co., London, England) operated at an accelerating voltage of 10 kV. X-ray and backscattered electron images as well as spot analyses were made with an electron microprobe analyser (Model 400-S, Materials Analysis Co., Palo Alto, California) operated at 20 kV accelerating voltage with 0.02 μ A sample current. Both secondary electron and X-ray emission images were recorded using Type 55 P/N Polaroid sheet film (Polaroid Corp., Cambridge, Massachusetts).

Comparisons between X-ray and secondary electron images

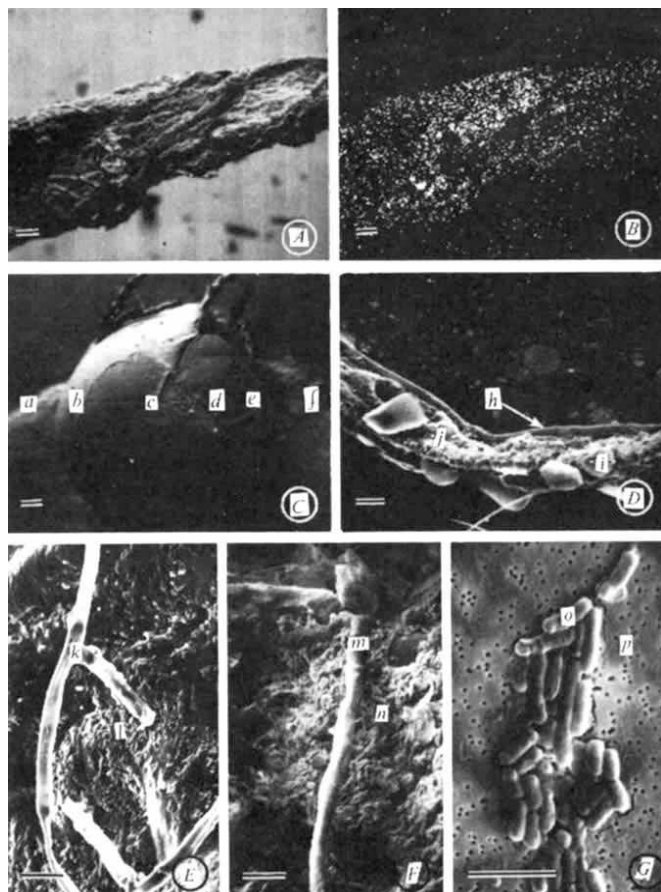


Fig. 1 *A* and *B* are electron microprobe analyser pictures of a rhizomorph sample isolated from decaying deciduous forest litter. A 100 μm marker is indicated at lower left. *A*, Image made by backscattered electrons. *B*, Calcium X-ray image. *C*, Galumnidae mite for which calcium values were determined after electron bombardment at each indicated point (*a* and *f* are background points) for 10 s periods at 20 kV. Calcium values (mg g^{-1}) for the points are: *a*, 1.5; *b*, 10.6; *c*, 18.9; *d*, 31.5; *e*, 12.7; *f*, 2.5. *D–G* are scanning electron micrographs of representative assay fields. *D*, White pine needle fragment (*g*) from litter with adhering insect wing scales (*h*) and a fungal hypha (*j*). *E*, Oak leaf (*l*) taken from decaying litter with a fungal hypha (*k*). *F*, Fungal hypha (*m*) in soil (*n*) underlying decaying oak litter. A 40 μm marker is indicated at lower left of each field. *G*, *Bacillus subtilis* cells (*o*) concentrated on the surface (*p*) of a membrane filter. A 10 μm marker is indicated at lower left.

were used to estimate the elemental content of a variety of biological specimens. These analyses have included a study of calcium, potassium, and magnesium distribution in organisms inhabiting decaying, mixed deciduous forest litter. Quantification of this technique is possible with macrochemical assay procedures such as emission spectroscopy and atomic absorption spectroscopy³. For example, the fungal rhizomorph illustrated in Fig. 1*A* and *B* contains $29.7 \text{ mg g}^{-1} \text{ Ca}$ as determined by these procedures. These counts can be converted to elemental values by comparison with standards of known concentrations. A calcium value of $134 \mu\text{g g}^{-1}$, potassium $194 \mu\text{g g}^{-1}$ and magnesium $140 \mu\text{g g}^{-1}$ is approximately equal to a respective X-ray emission value of one count every 10 s.

A quantitative application of microprobe analysis to biological material is illustrated in Fig. 1*A–G* and Table 1. Fig. 1*A* is a back-scattered electron micrograph of a rhizomorph sample, and Fig. 1*B* is a calcium X-ray image of the same rhizomorph. Fig. 1*C* depicts a whole body of a Galumnidae mite, a common litter-inhabiting organism which grazes on fungi. Point analysis counts, which resulted in minimum distortion of the sample, were made at each point along a transect (*a–s*).

An application of this technique which enables nutrients

to be analysed in a variety of biologically different samples located close to each other is illustrated in Fig. 1*D*. A freshly collected sample from the "L" layer of litter in a white pine stand was examined and comparison was made of the magnesium, calcium and potassium contents of a pine needle fragment, an insect wing scale and a fungal hypha, all situated within 1 mm (Table 1). All three samples contain the same relative amounts of magnesium and potassium. The calcium content of the insect wing scale, however, is eight times higher than that found in the pine litter. There is twice as much calcium in the fungal hypha as in the decaying pine needle.

Microprobe comparisons between a fungal hypha growing on a deciduous leaf and one growing in soil (Fig. 1*E* and *F*) are summarized by the numerical values for the magnesium, calcium and potassium content of these two samples in Table 1. There is no significant difference in the magnesium content of the fungal hypha colonizing the oak leaf or soil substrates. The calcium content of the fungal hypha is, however, twice that of the oak leaf, although there is no difference between the amount of calcium in the hypha and soil. Potassium seems to be concentrated within the fungal biomass of both microhabitats.

Chemical analysis of a specimen as small as an individual bacterial cell can be accomplished by the microprobe procedure, although samples of this size approach the $1 \mu\text{m}$ limit of resolution of the microprobe beam and are extremely "thin" with respect to the effective electron penetration. A stock culture of a common soil bacterium, *Bacillus subtilis*, was grown on a complex medium and the collected cells concentrated upon the surface of a 'Nuclepore' membrane filter⁹. Spot analyses were made of both the calcium and magnesium contents of the bacterial cells within the same field shown in Fig. 1*G* and are listed in Table 1. The calcium content of a *Bacillus subtilis* cell is estimated by the microprobe method as 1.1% and compares favourably with the 0.8% determined by emission spectroscopy for a mixed bacterial population grown under similar conditions³.

Electron probe microanalysis can be used to determine concentrations of essential nutrients in biological components of the terrestrial decomposer food web, such as the detrital substrate, microbial population and microbial grazers. As decomposer organisms are of great importance in cycling of nutrients in ecosystems¹⁰, any new technique which would give better estimates of nutrient pools contained within the decomposer food web would add substantially to the understanding of functional processes within ecosystems. Current modelling efforts being devoted to description and prediction of ecosystems by computer simulation would benefit from more accurate estimates of nutrient pools in decomposer organisms.

Several distinct advantages of this technique are evident when comparisons are made with existing macrochemical procedures. Examination of a variety of biologically different specimens located close to each other with minimum distortion of sample is possible. With the scanning electron microscope even a small specimen can be observed and uncontaminated areas can be selected for examination. Not only can estimates of microbial biomass be made from quantitative sampling techniques but it is also possible to ascertain the distribution of nutrients within the organism itself. As the specimen is not destroyed in the analytical procedure, assays of a given sample can be repeated to obtain statistical estimates of the nutrient content.

The application of this technique is not limited to investigations concerning terrestrial decomposer organisms, but will find application in any situation in which microorganisms play an integral part. The use of electron probe microanalysis will enable assessment of the role that decomposer organisms play in nutrient cycling.

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Table 1 Nutrient Analyses of Fungal and Bacterial Biomass shown in Fig. 1D-G

Specimen	No. of assays	Magnesium	Elemental content (mg/g) Calcium	Potassium
Fig. 1D				
Fungal hypha (h)	11	3.02 ± 0.32 * †	29.60 ± 2.48 *	22.36 ± 1.84
Insect wing scale (i)	6	2.47 ± 0.25	112.58 ± 14.84 *	18.95 ± 0.85
White pine needle (j)	7	1.72 ± 0.20	14.56 ± 4.31	18.37 ± 1.07
Fig. 1E				
Fungal hypha (k)	8	2.74 ± 0.25	81.64 ± 4.67 *	17.48 ± 0.46 *
Oak leaf (l)	5	3.30 ± 0.36	44.62 ± 8.21	13.23 ± 1.07
Fig. 1F				
Fungal hypha (m)	8	2.66 ± 0.43	66.07 ± 6.95	46.21 ± 2.81 *
Soil (n)	4	3.66 ± 0.98	65.15 ± 17.66	19.90 ± 7.46
Fig. 1G				
Bacteria (o)	12	0.08 ± 0.08	11.32 ± 2.41	No data

Values reported were determined from X-ray emissions after electron bombardment of each specimen for several 10 s periods at an accelerating voltage of 20 kV.

* Indicates significant difference ($P < 0.05$) in elemental content between sample and substrate (pine needle, oak leaf, soil).

† Standard error of mean is given for all samples.

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ROBERT L. TODD
KERMIT CROMACK
JOHN C. STORMER, JUN.

Institute of Ecology,
Department of Botany and Department of Geology,
University of Georgia,
Athens, Georgia 30601

Received December 27, 1972.

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Role of Protozoa in Waste Purification Systems

Pirt and Bazin¹ discussed possible adverse effects of bacterial predation by protozoa on the removal of substrate by activated sludge. Their analysis considered not activated sludge but a single-stage chemostat without recycle as used experimentally by Curds², and mathematically by Curds³ and Canale⁴, for the study of predator/prey interactions. Pirt and Bazin¹ concluded that efficient waste removal could best be achieved with two chemostats in series, eliminating protozoa from the first stage by operating it at a dilution rate in excess of their maximum specific growth rate.

Table 1 compares the concentrations of bacteria, protozoa, and substrate obtained from this system with those from a single-stage chemostat having the same overall residence time, demonstrating the better performance of the two-stage system. Table 1 also shows the effluent concentrations for a two-stage system of the same overall residence time which allows growth of protozoa in both stages. Contrary to the suggestion of Pirt and Bazin¹ the presence of protozoa in both stages improved treatment, with a further reduction in total solids concentration.

These results were obtained by assuming, as did Pirt and Bazin¹, that all the bacteria were dispersed and therefore

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Table 1 Calculated Concentrations of Bacteria, Substrate and Protozoa in Effluent from Single-stage and Two-stage Continuous Culture

Dilution rate (h ⁻¹)	Single-stage operation			Effluent concentration (mg l ⁻¹)		
	Specific growth rate (h ⁻¹)	Bacteria	Protozoa	Substrate	Bacteria	Protozoa
0.225	0.9989		0.225	8,858	128	221
Dilution rate (h ⁻¹)	Two-stage operation			Concentration (mg l ⁻¹)		
	Stage 1	Stage 2		Substrate	Bacteria	Protozoa
0.9	90	4,955	0	0.47	300	2,350
0.385	29.6	2,566	1,209	0.40	204	2,393

($\mu_{\max}$ bacteria = 1.0 h⁻¹, K_s bacteria = 10 mg l⁻¹, $\mu_{\max}$ protozoa = 0.4 h⁻¹, K_s protozoa = 100 mg l⁻¹, Y bacteria = Y protozoa = 0.5, S_0 = 10,000 mg l⁻¹, residence time 4.4 h).

available for predation⁵. Less than 10% of the bacteria in activated sludge are freely dispersed⁶, however, and those within the floc are relatively immune to predation. Fig. 1 shows the steady-state values obtained for the concentrations of bacteria, substrate, and protozoa in a single stage when another bacterium, using the same substrate but growing as a floc, is present. It was assumed that such a bacterium would grow at a slower rate than that of a dispersed organism, and the growth constants were chosen so that, in the absence of predation, it would be washed out of the system. In the three-component system washout of the protozoa occurred at the same dilution rate ($>0.39 \text{ h}^{-1}$) as in the two-component system¹, whereas the floc-forming organism was washed out when the dilution rate exceeded 0.38 h^{-1} . The presence of flocculated bacteria considerably reduced the effect of predation on substrate concentration (Fig. 1).

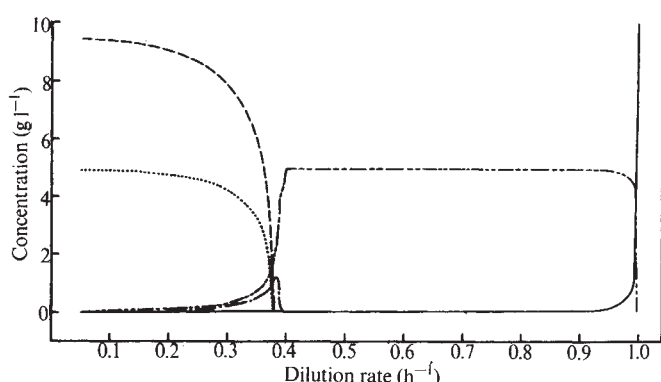


Fig. 1 Steady-state values of a prey, predator, and flocculated bacterium in a chemostat. Initial concentration of substrate, 10 g l^{-1} .

	$\mu_{\max} (\text{h}^{-1})$	$K_s (\text{mg l}^{-1})$	Y
Dispersed bacteria	1.0	10	0.5
Flocculated bacteria	0.5	20	0.5
Protozoa	0.4	100	0.5

Substrate (2-component system¹), ---; Substrate (3-component system), —; dispersed bacteria, — . . —; flocculated bacteria,; protozoa, — — — .

The requirement of waste treatment is to reduce the concentration of substrate (soluble organic compound or dispersed bacteria) as far as possible, thus limiting the extent of biological action when the effluent is discharged to the environment. It follows that within a biological process the microorganisms will be subject to severe substrate limitation and will have very low specific growth rates (Monod⁷; $\mu = \mu_{\max} s / (K_s + s)$). In the activated sludge process the critical stage is the settlement and recycling of the biomass so that the specific growth rate of the organisms is much less than the dilution rate^{8,10}, a situation similar to the second stage of a two-stage chemostat system¹¹ (Table 1). As settlement depends on the presence of flocculating bacteria, the activated-sludge process combines the advantages of a specific growth rate less than the dilution rate with the dominance of bacteria resistant to predation (Fig. 1). Additionally, attached forms of protozoa develop⁹ which settle and are recycled: these have a specific growth rate similar to that of the recycled bacteria and, for the same residence time, will reduce the concentration of dispersed bacteria in the final effluent to levels below those obtainable with the two-stage chemostat system. Thus, in the activated-sludge system the concentrations of both substrate and dispersed bacteria are reduced to a low level, the effect of predation on substrate concentration is minimized,

and the role of protozoa becomes chiefly that of effluent clarification⁵.

G. L. JONES

Water Pollution Research Laboratory,
Elder Way,
Stevenage,
Hertfordshire SG1 1TH

Received January 24; revised March 9, 1973.

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Resistance of *Raphanobrassica* to Clubroot Disease

THE classical amphidiploid *Raphanobrassica* ($2n=36$, genomic formula *rrcc*), first reported by Karpechenko¹, was of considerable academic interest and appears in many textbooks. As far as is known *Raphanobrassica* does not occur in the wild and it has not been exploited practically.

An attempt is being made at Pentlandsfield to develop *Raphanobrassica* as a new forage crop from autotetraploid forms of fodder radish (*Raphanus sativus* L., $2n=36$, *rrrr*) and fodder kale (*Brassica oleracea* L., $2n=36$, *cccc*). The possibility of clubroot resistance superior to that of rape (*B. napus* L. ssp. *oleifera* (Metzg. Sinsk.)) is an important consideration in this programme. That this might be possible is suggested by the fact that four cultivars of fodder radish proved completely resistant to four physiological races of the fungus *Plasmodiophora brassicae* Woron. isolated in Wales (Johnston, personal communication). Fodder kales are generally accepted as possessing at least some resistance, although little critical work has been carried out.

A number of *Plasmodiophora* cultures were obtained from farm crops in south and east of Scotland. Spores were extracted by the method described by Macfarlane² and stored at $2 \pm 1^\circ \text{C}$ in the dark. Spore numbers were assessed with the aid of a haemocytometer and diluted to a constant concentration of 10^7 spores ml^{-1} .

Various forms of *B. napus* (Table 1) have been used as differential hosts by Johnston³ and Paterson⁴ to identify the five biotypes of *Plasmodiophora* so far isolated in the UK. Seedlings of these differentials were grown to the cotyledon stage, their roots were dipped for 3 min in shallow dishes of spore suspension and they were then planted at $5 \times 5 \text{ cm}$ spacing in plastic gravel trays containing John Innes No. 2 compost (pH 6.2). Soil was kept uniformly moist and maintained at $20 \pm 3^\circ \text{C}$. Seedlings treated with different cultures were isolated from each other in separate trays. Air temperature did not fall below 8°C and plants were grown under a regime involving a "day" of 16 h.

Fifteen cultures were tested and varying reactions of the differential hosts obtained. Six cultures were found to infect heavily all five differential hosts.

Table 1 Reaction of *Brassica napus* and *Raphanobrassica* to Two Cultures of *Plasmodiophora*

<i>B. napus</i>	Numbers of infected seedlings in each infected class							
	Culture 1				Culture 2			
	None	Slight	Moderate	Severe	None	Slight	Moderate	Severe
"Giant" rape	—	—	—	10	—	—	—	10
Selection ex "Giant" rape	—	—	1	9	3	—	—	7
"New Zealand club root resistant" rape	2	—	—	8	2	—	—	8
"Wilhelmsburger" swede	—	—	3	7	2	—	—	7
"Nevin" rape	2	1	2	5	2	—	—	8
<i>Raphanobrassica</i>								
Family 1	10	—	—	—	7	2	1	—
Family 2	10	—	—	—	7	—	—	3
Family 3	10	—	—	—	9	1	—	—
Family 4	10	—	—	—	8	—	2	—
Family 5	10	—	—	—	10	—	—	—

In a second experiment, five F4 *Raphanobrassica* families were tested against two cultures giving the above reaction. These families were fairly closely related because sterility of F1 plants⁵ had much reduced the genetic base of subsequent generations.

Ten seedlings of each *Raphanobrassica* family and each differential host were treated. Seedlings were randomized in rows of five in each of two trays for each culture. After 6 weeks roots were scored for degree of club formation as an indication of the level of susceptibility. Results are shown in Table 1.

It is possible that cultures attacking all five differential hosts were actually aggregates of two or more races. It would require subculturing and/or a wider range of differentials to isolate these races. The fact that the *Raphanobrassica* families gave slightly different reactions to the two cultures tested is in accord with this premise.

Clubroot resistance in *Raphanobrassica*, not present in "Nevin", the most resistant rape cultivar commercially available, is clearly shown. The extent of this resistance remains to be explored but there are grounds for optimism that, should fertility problems be resolved, a useful new forage plant may eventually result.

I. H. McNAUGHTON

Scottish Plant Breeding Station,
Pentlandsfield,
Roslin, Midlothian EH25 9RF

Received March 19, 1973.

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⁵ McNaughton, I. H., *Euphytica*, **22**, 70 (1973).

and modern man (Table 1) indicates, however, that these two factors have a low correlation ($r=0.50$, compared to $r=0.98$ for the correlation between maternal and foetal (birth) weight of higher primates¹). This indicates that among higher primates the foetal growth rate is anything but constant, and therefore estimates on gestation periods based on this rate and birth weight are useless.

Table 1 Birth Weight and Gestation Period in Pongids and Modern Man*

Species	Mean birth weight (g)	Mean gestation period (d)
<i>Pongo pygmaeus</i>	1,500	275
<i>Pan troglodytes</i>	1,600	225
<i>Gorilla gorilla</i>	2,000	268
<i>Homo sapiens</i>	3,300	280

* Based on published data^{1,3}.

All that can safely be said about the gestation period of australopithecines is that its length had to be of the magnitude of that of pongids and modern man, and definitely shorter than 300 d as this has been proposed for *A. robustus*.

W. LEUTENEGGER

Department of Anthropology,
University of Wisconsin,
Madison, Wisconsin 53706

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Gestation Period and Birth Weight of *Australopithecus*

BASED on my estimates¹ of the foetal size at birth of *Australopithecus africanus* and *A. robustus*, Frazer² has calculated gestation periods to be 257 and 300 d, respectively. Calculations are based on the assumption of a constant foetal growth rate of 0.06 in higher primates. Regression analysis of gestation periods and birth weights in pongids

Reference Abbreviations

FROM September 1 it is *Nature's* intention that all abbreviations of references should conform to the style of the *World List of Scientific Periodicals*, fourth ed. (Butterworth, 1963-65). The changeover will be gradual and authors submitting manuscripts from now on are asked to ensure that the references are written appropriately.

BOOK REVIEWS

Monumental Prehistory

The Chambered Tombs of Scotland. By Audrey Shore Henshall. Volume 2. Pp. 656+38 plates. (Edinburgh University: Edinburgh, 1972.) £15.

WITH the publication of volume 2 of *The Chambered Tombs of Scotland* Audrey Shore Henshall has brought to fruition nearly two decades of intensive survey and study. These tombs undoubtedly rank as the most numerous and impressive instances of early architecture in Britain: although some 5,000 years old, a number of them still stand virtually complete, and can be viewed today in a condition very close to that in which they were left by their makers, which is probably more than can be said for any Roman or Saxon structure in these islands.

The tombs discussed in this second volume, which covers western and southern Scotland, are two hundred in number. They are divided into three groups (Bargrennan, Clyde and Hebridean, plus long cairns), supplementing the six groups in northern and eastern Scotland discussed in volume 1. They are listed systematically by county, each with a plan (often by the author herself), with a full description and, where the gravegoods are preserved, with drawings of all the finds. This is undoubtedly one of the most monumental achievements in the whole field of British archaeology: it will still be an indispensable work of reference a century hence.

For the scholar today the interest does not lie solely in the great mass of data presented in the corpus. Miss Henshall's discussion of the origin and development of the tombs has been eagerly awaited, particularly because the full impact of radiocarbon dating has been felt since the publication of her first volume. The longer chronology now makes clear that many of these tombs are composite monuments, the result of several phases of construction and of reconstruction. This realization makes the question of origins a little clearer, and it is no longer necessary to seek a separate overseas origin for each of the nine groups identified. An early phase of building is now suggested in south-west Scotland, before 3000 BC, related no doubt to analogous developments in Ireland, with small rectangular burial chambers inside small cairns. In phase two the first passage graves—simple polygonal chambers with an entrance

passage—were built. The story from then on is one of increasing local elaboration culminating in the construction of the impressive tombs of Caithness and Orkney, where the masterpiece of Scottish neolithic architecture, Maes Howe, may reflect further contacts with Ireland.

Some elements of this sequence are hypothetical, but the important and progressive shift is to see these developments in essentially local terms, although not divorced from a broader European context. The way is now open for more sustained locational studies, rather than the detailed typological treatments which have sometimes in recent decades become too elaborate.

Miss Henshall rightly emphasizes that in nearly all cases the gravegoods found in these tombs relate only to the final phases of their use, often centuries after their original construction. In many areas we are left with the paradoxical situation that the only archaeological evidence from the makers of these impressive monuments lies in the tombs themselves, with no artefacts or indications of domestic settlement to supplement them. It is to be hoped that this is a situation which archaeology will eventually rectify. But meanwhile we are indeed fortunate that the tombs themselves are presented here so comprehensively. The Edinburgh University Press has again produced the volume very handsomely.

In his foreword to the first volume, Professor Stuart Piggott hailed "Miss Henshall's achievement . . . a major and outstanding contribution, of solid and enduring worth, to British prehistoric studies". The publication of the second volume confirms this verdict: it is surely the most substantial contribution made for several decades to the study of British prehistory.

COLIN RENFREW

Reptiles and Amphibians

Herpetology. By Kenneth R. Porter. Pp. xi+524. (W. B. Saunders: Philadelphia, London and Toronto, 1972.) £6.60; \$15.50.

PROFESSIONAL herpetologists have long despaired of there ever being published an authoritative text that would meet their needs. The high standard achieved by the contributors to the recently published volumes in the *Biology of the*

Reptilia and the informative *Life of the Reptiles* has gone a long way towards satisfying workers on that class of vertebrates but those concerned with amphibians still lacked any textbook more recent than Noble's 1931 *Biology of the Amphibia*. Kenneth Porter's *Herpetology* seems destined to be the book that has been hoped for. It has remarkably wide coverage and reflects organizing skill and a prodigious amount of labour in sifting the relevant literature, yet the mass of information is contained in a manageable size.

The contents include sections on origins and phylogeny with generous reference to palaeontological material and characterizations to family and sub-family level, zoogeography, moisture and temperature relations, colour change, feeding, isolating mechanisms, population dynamics and man's interactions with reptiles and amphibians; about two hundred pages are devoted to functional morphology and reproductive adaptations. A list of references follows each section.

The systematic treatment follows conservative lines and generally incorporates the arguments and the changes that have been presented by the leading workers in the field but surprising omissions are both McDowell's and Miller's convincing evidence that the Dibamidae and the Anelytropsidae are congeneric and Gasc's discussion of the family's affinities. But on the whole there are reasonably few errors of omission and the text is commendably free from typographical errors. The subject index would benefit from expansion: I had to engage in mental gymnastics tracking down topics such as the lateral line system and autotomy. Another minor defect that will no doubt be rectified in a second edition lies in the use of illustrations reproduced from books originally published many years ago with captions bearing out of date names that appear nowhere in Porter's text.

It is sobering to find in the section on leading centres and British herpetologists that the author rates highly only three workers, two of whom are dead. Despite liberal quotations from G. L. Underwood's and I. Griffiths's works, the British nationality of these authors is not acknowledged. But not even this slight can detract from the value and usefulness of this highly scholarly treatise which can be enthusiastically recommended to all who are interested in herpetology. A. G. C. GRANDISON

A Model of Development

Science Policy and Development: The Case of Israel. Edited by E. Tal and Y. Ezrahi. (Israel-Latin American Symposium on Science Policy and Organization of Research, held in Israel, January 1970.) Pp. 353. (National Council for Research and Development: Jerusalem; Gordon and Breach: New York and London, 1972.) n.p.

THIS volume is a collection of twenty-two main papers delivered at the Israel-Latin American symposium in 1970, plus an extract from the discussion. It suffers from some of the usual defects of such a volume, notably the absence of an index and the unevenness of the contributions which range from over-familiar generalities about science policy to important specific evidence of Israeli experience.

Israel commands special attention from all interested in science policy. She provides a model for those starting science-based industries from scratch, and is endowed with a promising new government organization, the National Council for Research and Development, set up after Professor Katchalski's¹ enquiry of 1966-68. But Israel has also managed to look outward, and help the developing states with her knowledge and skills, though recent experiences in Uganda and in Chad may have been discouraging.

Yet Professor Sabine's view of Israel in his keynote speech at the symposium as "an important pilot plant for the developing countries of the world" claims too much. Even allowing for Israel's special difficulties, these other countries do not have the human and educational resources, the important overseas connexions and the sheer abilities that make Israel unique. Professor Sabine himself reveals he is only a "recent" Israeli, coming from the US, and elsewhere Professor Keynan says 80 per cent of the Hebrew University's research budget comes from abroad. Professor Bergmann indeed emphasizes that the characteristic difference between Israel and other countries is that she had a scientific establishment long before she had a political establishment. Dr Arnon says firmly that Israel cannot provide recipes for other countries: "every country must solve its own problems".

Many of the contributions deal with universal problems: government scientific organization; government finance for research and development; the role of scientists in government and management; the evaluation of research; the argument for independent scientific research even in small countries; industrial attitudes to research; the relationships between pure and applied research, between government and industry and between universities and industry. On

this last item, the influence of the Weizmann Institute and its "industrial park" is especially interesting. The role of defence in science policy is, unfortunately, only touched upon; this is clearly a field where a detailed account of Israel's experience would be illuminating.

The most rewarding chapters, for me, are those in the middle, which deal with agriculture and science-based industry. Dr Arnon's two chapters on agriculture are analytical and practical, lively and illuminating. He cites the development of Arab agriculture in Israel as something really relevant to developing countries, for it shows how knowledge of improved technology *per se* and even the availability of improved inputs has no influence whatsoever on traditional agriculture until a whole set of circumstances change. The industrial case studies are also most interesting, while Mr Tolkovsky's paper on private investment in science-based industries stands high for its wisdom and practical experience.

The volume as a whole reflects the vitality of science and industry in Israel. If too little is said of Latin America, we may take it that the participants from those countries were more anxious to listen than to speak. If so, they would have learned much.

MARGARET GOWING

¹ Professor Katchalski is now President of Israel.

Seeing Infrared

Laboratory Methods in Infrared Spectroscopy. Edited by R. G. J. Miller and B. C. Stace. Pp. xxi+375. (Heyden: London and New York, September 1972.) \$18; £6.25.

THIS is the second edition of a well-known book on the experimental techniques associated with infrared spectroscopy. The infrared method is a very powerful one for structural determination and, in suitable instances, for quantitative analysis. In large measure, its power is to be found in its capability for dealing with an extremely wide range of types of samples—from gases to crystalline solids and from fibres to pastes. In many cases points of experimental technique make the vital difference between success and failure in this type of work and, despite a 25-year period of development, considerable progress continues to be made.

The coverage in topics in this second edition is considerably wider than before and, with the exception of the omission of a specific chapter on infrared emission spectroscopy, is really comprehensive. It includes also chapters on Raman spectroscopy and Fourier transform infrared spectroscopy. The same care as before has been shown in choosing

contributors who are highly experienced in the various techniques, and the editors have been skilful in guarding against unnecessary overlap in the different contributions. It is a pity, however, that there is no index.

Without question this is the best as well as the most up-to-date account of infrared experimental techniques for the chemical spectroscopist. It is very highly recommended and deserves a wide sale.

N. SHEPPARD

The Purines

The Purines: Theory and Experiment. Edited by Ernst D. Bergmann and Bernard Pullman. (Proceedings of a symposium held at Jerusalem, April 1971.) Pp. 61. (The Israel Academy of Sciences and Humanities: Jerusalem, November 1972.) (Distributed by Academic: New York and London.) \$29.

Regulation of Purine Biosynthesis. By J. Frank Henderson. Pp. xv+303. (American Chemical Society: Washington DC, 1973.) \$12.95.

THE first of these books presents a remarkable instant picture of the state of knowledge of purine structure and chemistry at the beginning of the 1970s. By their success in drawing together the majority of outstanding workers in this field, and asking them to present a contemporary contribution to the theoretical and experimental aspects of the purines, the organizers of the symposium have captured, and preserved for society in this one volume, a fascinating collection of forty-eight original papers. There was apparently no division into subject areas during the symposium but a subsequent examination reveals four main areas into which the bulk of the papers could be placed: (1) The molecular structure of simple purines, purine adducts, nucleosides and nucleotides; (2) properties of the purines, for example, tautomerism, site of protonation and metal attachment, photochemical reaction; (3) the organic, biochemical, and medicinal chemistry of purines and related compounds; and (4) papers with a predominantly theoretical content, in particular quantum theory and molecular orbital calculation. The application of physical and physicochemical methods for structure determination is essential to current work and the supreme importance in this field of X-ray crystallography was shown by the fact that more than one quarter of the total papers were largely concerned with this technique. In addition several papers referred to i.r., u.v., n.m.r. and fluorescence spectrometry and electrical conductivity, e.s.r., mass spectrometry, and circular dichroism.

Undoubtedly the first of the above subject areas is the one at present un-

dergoing the most rapid rate of increase in knowledge and the one in which the most exciting papers appeared. To single out any small number of papers must be somewhat invidious, but four of the most fascinating to a reader of the book are "The Structure of Adenosine Triphosphate" by the Cambridge team of O. Kennard, W. W. Isaacs, W. D. S. Motherwell and D. G. Watson, "The Stereochemistry of Actinomycin Binding to DNA" by Henry M. Sobell, "Solid-State Stacking Patterns of Purine Bases" by C. E. Bugg and "Conformations in Polynucleotides" by S. Arnott.

All the papers were submitted to a critical but appreciative audience and it is one of the merits of the book that the discussion is included. Particularly relevant criticisms to many of the papers came from M. Sundralingam and W. Pfeleiderer and two recurrent themes discussed on several occasions were the forces controlling base stacking and the positions of protonation in several related purines. The two editors, E. D. Bergmann and B. Pullman, were themselves authors of papers which linked theoretical propositions with practical and they are to be congratulated for their editorial work.

The book is well produced with clear formulae, tables, diagrams and photographs, but the index is somewhat short for a book of this size. A final chapter entitled "Concluding Remarks", by E. D. Bergmann, is useful for a rapid check of the main theme of each paper and the summary which several authors give could with advantage have been made mandatory for all.

Regulation of Purine Biosynthesis is a stimulating and exciting book which should be read by all those interested in purine chemistry and in the study of structural problems in heterocyclic chemistry in general. The *Chemical Monographs* published by the American Chemical Society since 1921 are intended to make available to chemists a thorough treatment of a selected area in a form usable by persons working in more or less unrelated fields to the end that may correlate their own work with a larger area of physical science and to stimulate further research in the specific field treated. Professor Henderson has undoubtedly been thorough in his review of the biochemical literature for the production of the monograph *Regulation of Purine Biosynthesis*. In a final chapter, however, he is inevitably brought to the conclusion that practically nothing is known of how regulatory mechanisms operate in intact cells and how the different regulatory mechanisms for the discrete steps are integrated. It is furthermore obvious from reading the book that very little is known of the mechanism at the molecular level of each of the eleven steps in the bio-

synthetic pathway from inosine-5'-phosphate to inosinic acid.

Possibly because the author attempted to refer to so many original papers the book seems at times to be a catalogue of summarized findings from other workers. One would have welcomed a more critical approach and a call for help from theoretical chemistry, X-ray crystallography, ^{13}C n.m.r. spectroscopy and other potentially useful areas. There are several printing errors, particularly in the formulae, and pyrimidinones, pteridinones, purinones and mercaptopurines are all incorrectly represented in their unlikely tautomeric structures.

Regulation of Purine Biosynthesis shows how too narrow an approach by very many workers has not answered important biochemical questions. The Jerusalem symposium demonstrates the types of tool which are effecting major advances in other biochemical areas and which must surely be valuable in the solution of all heterocyclic biochemical problems.

D. G. WIBBERLEY

Neutral Mutations

Theoretical Aspects of Population Genetics. By M. Kimura and T. Ohta. Pp. ix+219. (Princeton University: Princeton, New Jersey, 1971.) \$12.50.

THIS is an excellent book, which should be read by everyone interested in population genetics. The authors are two of the world's outstanding theoretical geneticists, and this book is essentially an account of their contributions to the field over the past five years or so. It is admirably clear and concise; the non-mathematically minded are catered for by the relegation of most of the mathematical proofs to an appendix. In chapter 1, the problem of the fixation of mutant genes is treated; this is used in chapter 2 in discussing the interpretation of observations on the rate of evolution as measured from protein sequence data. Later chapters deal with the concept of effective population size, the theory of genetic load, two-locus problems, the maintenance of variability in populations, and the adaptive significance of sex.

Kimura and Ohta believe that most nucleotide substitutions in evolution are due to the random fixation of selectively neutral alleles, and that protein polymorphisms revealed by electrophoresis merely represent a transient phase of this molecular evolution. Most of the mathematical results described in this book have been developed in order to show that this theory can account for the known facts of protein variation and evolution. Kimura and Ohta do a very good job of presenting this view, and only the most dyed-in-the-wool pan-

selectionist can fail to be impressed. Nevertheless, there are some facts which are hard to fit into a neutral scheme of things, notably Prakash and Lewontin's discovery of non-random associations between inversions and protein variants in *Drosophila pseudoobscura*. It is a pity that these observations are not discussed in this book.

Many criticisms have, of course, been levelled against the authors' arguments for neutral mutations. In this book, most of them are dealt with quite convincingly. There does, however, seem to be an inconsistency which is hard to overcome. Kimura and Ohta show that, on the neutral mutation theory, the rate of amino acid substitution in evolution is equal to the rate of origin by mutation of new alleles affecting protein structure. If the data from proteins which have been sequenced are taken as representative, the average rate of mutation to neutral alleles per locus per generation can be computed. Kimura and Ohta find that this rate is less than one-tenth the order of magnitude of the mutation rates per locus measured experimentally in higher organisms. These mutation rates are based almost exclusively on rates of mutation to deleterious alleles. They therefore conclude that "this suggests that the neutral mutations constitute a rather small fraction of the total mutations".

Now the rate of neutral mutation for the whole genome can be estimated by multiplying the rate per nucleotide site by the total number of sites in the genome, calculated from the DNA content of sperm. For man, Kimura and Ohta estimate that between sixty and seventy-five neutral mutations occur per genome per generation. They argue that this means that "nucleotide substitution has an appreciable effect on fitness in only a small fraction of DNA sites", otherwise "the mutational load must be unbearably high for human populations".

This is a puzzling contradiction, which is difficult to resolve without serious damage to some other parts of the case for neutral mutations. For example, if one assumes that much of the DNA is functionless, the argument that most amino acid substitutions cannot be adaptive (because there would otherwise be too high a substitutional load) loses its force. Perhaps Kimura and Ohta would argue that observed mutation rates are biased in favour of loci which are known to have mutated and which therefore must have higher than average mutation rates. This can be tested by measuring mutation rates for electrophoretically detectable loci. Some data of this sort have been published by Mukai and by Kojima, and these agree quite well with usual mutation rates.

I would emphasize again that this is

an important and well-written book. The question of the possible selective neutrality of most protein variation is one of the most interesting in contemporary biology. Kimura and Ohta have greatly refined the mathematical tools needed for tackling this problem, and have made us all much more critical in our attitude towards evidence in favour of selection. This book is a valuable account of their work.

BRIAN CHARLESWORTH

First Know Your Rocks

Petrology of the Igneous Rocks. By F. H. Hatch, A. K. Wells and M. K. Wells. Rewritten thirteenth edition. Pp. 551. (Thomas Murby: London, April 1973.) £7.40.

MOST students of petrology will be familiar with "Hatch and Wells", which has enjoyed a long and honourable career as a student textbook since its first appearance in 1891. Even those nurtured on the rival "Harker" were (and indeed still are) advised, when sufficiently mature, to consult this work for its excellent account of igneous activity in the British Isles, although they may have been firmly directed to read no further. In this book the emphasis has always been placed firmly on a clear interpretative exposition of petrography based on evidence that can be gleaned from the rocks. Despite the esoteric temptations offered by some modern treatments or by the *art nouveau* of plate tectonics, the Wells family has adhered firmly to its belief that any appreciation of the global setting of petrology can only be achieved properly if the student first knows his rocks.

During the twelve years that have intervened between the twelfth and thirteenth editions, our knowledge of the evolution of the Earth's crust has been completely transformed: no less dramatic have been the advances in the fields of experimental petrology and geochemistry which have enabled a degree of certainty, hitherto lacking, to be given to petrogenetic theory. It is clear that these changes have been appreciated by the authors, although they have not exactly been swept off their feet. Although much of the otiose crystallographic material has been removed, surely it is petrographic mineralogy that is needed; all the structural mineralogy should have gone as well. A chapter on the geological setting of igneous activity has been added and those dealing with basalts, andesites, trachytes and rhyolites have been completely revised and extended. A new approach has been made to the vexed problem of rock classification and nomenclature. Although a satisfactory classification of salic rocks is presented,

no clear scheme is apparent for the intermediate and mafic varieties; a comprehensive classification table should have been included. New material has been added to other chapters where needed and references have been updated. All this has been achieved without substantially increasing the size: alas, the same cannot be said of the price.

In view of the extensive rewriting, it is a pity that the opportunity was not taken to increase the page size, for the book does not lie open well. References could have been found more easily and the appearance would have been improved if the numerous footnotes had been replaced by a full bibliography. It is pleasant to note that the fine micro drawings have been retained; these portray so much more clearly than do photomicrographs the textural features of the rocks. The treatment of chemical data is less satisfactory, for although many tables of rock analyses are included and the principles of both the CIPW Norm and Niggli values are explained, they are little used. Variation diagrams are treated very briefly, and although simple phase diagrams are introduced early in the book they are rarely used to explain the petrology.

In spite of these criticisms the authors deserve to be congratulated on presenting a well balanced, interesting and thoughtful modern account of igneous petrology written for the level of a second year student.

I. D. MUIR

Interstellar Scatter

Light Scattering Functions for Small Particles with Applications in Astronomy. By N. C. Wickramasinghe. Pp. 506. (Adam Hilger: London, 1973.) £12.

AFTER a short summary of observational evidence for interstellar dust and the extinction of electromagnetic radiation by that dust, followed by a similarly short account of the computational procedures used to calculate the efficiency factors for scattering, absorption and back-scattering and the forward directivity of scattering of spherical cylindrical and composite dust grains, the book goes on to detail these factors over a wide range of refractive and absorptive indices and radius of particle in wavelength units. Graphs of the results are given for selected cases. Specific computations are given for ice, iron and graphite and for composites with graphite and iron cores.

From this brief description it will be obvious that this is a book for the aficionado, even if these should include students of meteorology and colloid science, as claimed. It is a pity that computations were not given for the

currently favoured silicate material. Nevertheless, the book covers a very wide range of conditions and will be useful both for itself and as a spur for others contemplating similar computations.

The book is well and attractively produced, the tables being of tidily laid out computer output. Its main fault is the price, surprisingly high for a book of over 90% of photolitho content. The user will probably borrow his copy from his library, which ought to have one.

H. SEDDON

Hyperfine Structure

Theory of Hyperfine Structure of Free Atoms. By Lloyd Armstrong, Jr. Pp. ix + 209. (Wiley: New York and London, March 1972.) £7.

TECHNICAL progress in atomic spectroscopy in the last twenty years, the development of highly monochromatic sources of radiation such as atomic beam frequency standards, masers and lasers, and sophisticated spectrometers capable of detecting transitions between individual atomic states, has allowed a great increase in the precision with which hyperfine interactions in free atoms can be measured. It is not surprising that there has been a corresponding development in the rigour and complexity of the theory to such an extent that it is now difficult for an active research worker to follow developments without reference to the original literature associated with several diverse fields. This volume represents what is probably the first comprehensive review of the theory of fine and hyperfine interactions. The interactions between the nucleus and the electrons are analysed using a consistent set of techniques and clearly defined approximations in such a way that the relationship between the theory of hyperfine interactions and the theory of atomic fine structure is stressed. The basic theories of angular momentum, second quantization, relativistic electrons and Lie groups are presented in the first place and then used to develop the theory of atomic structure and the hyperfine interaction. There is one chapter on the effects of external electric and magnetic fields and another concerned with higher order effects such as the hyperfine anomalies associated with nuclear structure and electric dipole moments. The final chapters deal with the hyperfine interactions in one electron and many electron atoms. The presentation is too sophisticated for the average experimentalist who will probably find some of the techniques used unfamiliar and difficult, but the volume will be of great help to the research worker and the graduate student who needs to study the subject in depth.

K. E. SMITH

CORRESPONDENCE

Weber's Waves

SIR,—I feel impelled to write about the report on the Oxford–Cambridge–London Relativity Seminar (*Nature*, **243**, 61; 1973), as the account contains several inaccuracies and might give someone unfamiliar with the current experiments on gravitational radiation some misleading impressions. The reporter seems to have drawn the conclusion that it has already been shown that Weber's gravitational wave detectors are not detecting gravitational waves when in fact this degree of finality goes rather beyond the implications of currently available experimental data. It is true that Weber's experiments have not been confirmed, and indeed the first results from three or four experimental groups make it appear more probable that Weber's interpretation of his data is mistaken than that it is correct; but I do not think that experimenters working in the field have claimed this was a definite conclusion yet, and it seems rather premature for the *Nature* reporter to assume it and incorporate it so strongly in the title of his article.

As the report mentions a review which I gave at Oxford of the current experimental situation, I think I should point out that some of the statements which might appear to be attributed to myself are inaccurate and do not accord with my own views. For example, it is reported that all gravitational wave detectors are cooled to reduce thermal effects—when, although cooled detectors are being built, few, if any, are in operation. And I do not think that the conclusion is inescapable that Weber has not detected gravitational waves. Experiments in this field are long and difficult, and although one may speculate about the probable outcome I think it is too early to make definite conclusions at this stage.

In several places in this article, including the report of the theoretical discussion, I feel there are slight inaccuracies which, although small in themselves and probably accidental, do give an overall impression which may not help the research and may indeed unnecessarily hurt some of the persons involved. Although research on gravitational radiation is of a type which may, or may not, uncover new and unpredicted

phenomena, I do not think it helps to overdramatize some aspects of the situation when what is required is patient experimental work together with careful interpretation.

Yours faithfully,

R. W. P. DREVER

*Department of Natural Philosophy,
Glasgow University, Glasgow G12 8QQ*

The Mind Diseased

SIR,—Dr Thomas S. Szasz (*Nature*, **242**, 305; 1973) writes that, strictly speaking, "... disease or illness can affect only the body. Hence there can be no such thing as mental illness". Thus he assumes that mental and bodily functions can be clearly separated, and that the mind is something apart from the body. This appears untenable in the light of modern biology; the mind cannot be divorced from the organism as a whole. Dr Szasz appears further to say—although I wonder whether he really means it—that "mental patients", who suffered from no recognizable bodily illness, were generally and properly considered to be fakers and malingerers, until Charcot, Janet, and Freud led us astray with the concept of mental illness. I believe that Dr Szasz is wrong, both philosophically and historically.

No doubt many patients in the past were considered malingerers. Before the functions of the thyroid were understood, for instance, victims of hyperthyroidism or myxoedema were probably often placed in this category. Our knowledge of the finer details of the working of the brain is still rudimentary; the psychiatrist, dealing with a patient who is suffering agonizing distress, may not be able to put his finger on any objectively detectable abnormality in the patient's nervous system or hormonal balance. Are we therefore to say that the patient is not really ill, because no physical or chemical test is yet available, to be correlated with the symptoms? Does illness become real when someone discovers such a test, although it was unreal before?

The idea that nobody before Charcot believed in the reality of mental illness

is untenable. Consider, for instance, the cry of Macbeth to the doctor who is treating Lady Macbeth: "Canst thou not minister to a mind diseased?" In this case, certainly, the doctor was helpless, but it is obvious that both Shakespeare and his audience believed in the reality of mental illness.

One famous eighteenth century patient, William Pitt, Earl of Chatham, suffered from occasional manic episodes and from several long periods of terrible depression, when he was completely incapacitated for many months at a time. During one such period (1767–68) Pitt was nominally Prime Minister; he begged George III to let him resign, because he was ill, and the King finally and reluctantly granted his request. Pitt's physician, Anthony Addington, certainly considered the depression as an illness; his major therapeutic approach was to encourage the development of attacks of gout, a disease to which Pitt was also subject. This was perhaps the eighteenth century equivalent of shock therapy. In any case, both Pitt and his doctor regarded these depressions as real illnesses¹.

Dr Szasz proposes that all those who commit crimes should be dealt with by criminal law. Perhaps; but there is a difference between the man who kills another to get his money, and one who kills because he believes that his victim was trying to destroy him by evil magic. Perhaps both should be tried by the same judicial procedure, but surely the sentencing of the criminal should take the particular circumstances into account.

I share the concern of Dr Szasz over the outrages inflicted on many patients confined in mental hospitals against their will. A campaign against such cruelty and injustice is entirely in order, but I believe that it will have a better chance of success if it is not entangled with a highly dubious philosophical outlook.

Yours faithfully,

JOHN T. EDSALL

*Biological Laboratories,
Harvard University,
16 Divinity Avenue,
Cambridge, Massachusetts 02138*

¹ Tunstall, Brian, *William Pitt, Earl of Chatham* (Hodder and Stoughton, London, 1938).

Eddington

SIR,—The letter you published by H. W. Grayson (*Nature*, **242**, 317; 1973) must, on internal evidence, be judged to be fraudulent: the strong implication of a pre-1926 date in the letter does not agree with a reference to Sir Arthur Eddington—Professor A. S. Eddington was awarded a knighthood in 1930.

Yours faithfully,

H. MYKURA

*School of Materials Science,
Physics Building,
University of Warwick,
Coventry, Warwickshire CV4 7AL*

Complementarity

SIR,—The book review by E. H. Hutten, "Microphysical Phenomena" (*Nature*, **241**, 220; 1973), suggesting the need for a new approach to such problems after fifty years of discussion, has just come to my notice.

Complementarity is invoked, but it is the principle of interdependent incompatibility which needs to be applied. According to this principle, the co-existence of mutually exclusive states is possible when each state determines the other and is thereby instantaneously excluded (like the concepts "position" and "displacement from that position", for instance), so that the two states alternate so rapidly within any given period of time that they appear to co-exist at the same time.

The Greeks worried about this problem, but notoriously never made experiments. Now, some 2,000 years of test results later, it pays to look once more at the problems they posed, in the light of these results.

I have postulated the principle of interdependent incompatibility not as an abstruse hypothesis, but because it is simple, obvious and fits the observed facts better than any other—as I could show, given the chance, or as could be worked out by others who put their mind to it. So far, I have hawked the principle variously, in vain. What good is the demand for a new approach if, when such approach is made, no one wants to know?

Yours faithfully,

U. LIGHT

*65 Upper Queens Road,
Ashford, Kent*

Fossils of Creation

SIR,—I feel that I really must argue with E. C. Lucas (*Nature*, **242**, 355; 1973) on his suggestion that fossil evidence supports the Hebrew creation myth. The old ideas of the "ages" must be dropped; the age of fishes, the age of the coal forests and so on, have no reality.

Fishes appear in the Devonian but must have had a long evolutionary history which has not been preserved. Reptiles and mammals appear in the Perino-Trias, again fully evolved. One cannot interpret the six days of creation in terms of artificial geological epochs; it is taking credulity too far and is interpreting "Neolithic science" in terms of modern science. Why six days? The Neolithic farmer would have known that the phases of the Moon repeated every 28 days. His natural submultiples of numbers would be obtained by halving. The smallest whole number which would be obtained by repeated halving is seven. Seven days becomes a week. Four weeks becomes a month (lunation), thirteen lunations becomes one year of 364 days. Thus he had seven days to the week, four weeks to the month and thirteen months to the year. The "science" of numerology led to giving magical significance to these numbers. I need elaborate this argument no further.

The Neolithic farmer would work for six days and worship on the seventh because the seventh day was "magic". Naturally he would extend such reasoning to the creator who would labour for six days on his creation. As to the order of creation he would start at the "bottom" and work his way up to the "top". The ocean, the land, the heavens. Man, naturally, would occupy a special phase as the last and most perfect of all the creation.

The *Bible* is a collection of stories, songs and legends not necessarily in the correct chronological order. The *Bible*, like the *Iliad*, or Geoffrey of Monmouth's history of the Kings of Britain, contains a strong element of historical fact (or should it be inference?) which we must be careful not to interpret too literally.

I am sorry, but the Hebrew creation myth has no support at all from the fossil record. If Mr Lucas wishes to believe in it then he must evoke an act of faith—a highly unscientific mental process.

Yours faithfully,

J. SAXON

*7 Rockwell Terrace,
Thurso,
Caithness*

Games with GNP

SIR,—Although grateful to *Nature* for printing my paper "Features of a Closed System Economy" (*Nature*, **242**, 561; 1973), I should point out in connexion with the editorial, "Fun and Games with GNP", that Boulding's writing, and especially his books, *Economics as a Science* and *Beyond Economics*, in fact led to my paper, and in both the professor is apparently guilty of the "curiously

subjective" error which I am accused of, because he roundly equates GNP to gross national cost with, I think, rather brilliant reasoning based on thermodynamics. My question of gross product to the sum of gross consumption (and the rest used to improve society) is a precise quotation from the professor's book *Economics as a Science* (page 45).

I have gone further than did the professor in analysing "good" and "bad" costs but the idea is his. There is also no difficulty on real cost. The real cost of an activity is the amount of non-renewable resource converted by it from potential energy in the ground to useless and irrecoverable waste (plus the personal effort involved in it). It is obviously akin to entropy in physics although essentially a vector rather than a scalar quantity. The difficulty of avoiding some arbitrariness in drawing the line between activities which obviously increase entropy and those which decrease it (Boulding's negative entropy of "structures of increasing improbability") is because we are dealing with a social science and not physics. But difficulties of definition do not destroy realities.

The illustration given about education is mistaken, and does not invalidate Boulding's argument (or mine). Clearly the contribution of policies towards discouraging the kinds of investment which merely add to further wasteful or unnecessary consumption must in the end be to limit the supply of unnecessary goods—and the consuming public (including the school teachers) can only buy what is available. The difference is clear enough, I think, between the likely behaviour pattern of a potentially rational society and the situation of the artificially stimulated captive consumers so well described in Vance Packard's book, *The Waste Makers*.

The technological optimism of the last paragraph is, I believe, of the wrong kind, and here I draw attention to recent published work of the US Geological Survey relating to energy resources for power production. This makes it clear, I think, that one of the risks of absolute or near shortages could be increasing rigidity, as substitution technology is invariably power hungry. From this kind of thinking I would guess that the oceanic uranium referred to will probably remain where it is! I believe the true direction of future technology is towards more advanced conservative systems as suggested in the last part of my paper, to which no space or regard is given; this, along with quite far-reaching changes in values and habits of mind, which are at present almost hopelessly one-tracked. And are we so sure that we are on the right track, in looking at the possibilities of tearing the Earth apart merely to sustain a high level of activity and a rapidly ageing body of dogma

which many economists seem to believe in with almost incredible persistence?

Finally, on relevance to the real world. First, the exercise has to do with the need for major regional projects of the Russell-Spoffard kind on the European side to at least provide the infrastructure of more advanced systems should the cultural difficulties ever be overcome; second, for greater investment in recycling and recovery projects of the kind currently undertaken and publicized by the National Research Development Corporation; and third, it clearly concerns changes in design thinking by the engineering industries, in the direction of durability and reducing obsolescence.

Yours faithfully,

R. E. OVERBURY

37 Eamont Court,
St Johns Wood,
London NW8

Physical Units

SIR,—An article under the title, "Natural Units in Atomic and Molecular Physics", from Professor R. McWeeny which appeared in your issue of May 25 (243, 196; 1973) draws attention to an area in which there is need of common international usage. It has already been announced that the IUPAC Commission on Physicochemical Symbols, Terminology and Units might consider this question and opinions and suggestions have been requested by its Chairman, Dr M. A. Paul, NRC Division of Chemistry and Chemical Technology, National Academy of Sciences, Washington DC 20418, USA. The need for an authoritative examination and agreed recommendation is brought out by McWeeny's article since most scientists feel that if letters such as H, as, aV and aT have internationally agreed meanings as the Henry, attosecond, attovolt and attotesla, it is objectionable for them to have special meanings of hartree, atomic second, atomic volt and atomic tesla in special contexts. What is one's reaction to the statement $1 \text{ as} = 24.1889 \text{ as}$?

Yours faithfully,

D. H. WHIFFEN

Department of Physical Chemistry,
The University,
Newcastle upon Tyne NE1 7RU

Announcements

International Meetings

July 1–7, **The Study of Time.** (Secretary, J. T. Fraser, International Society for the Study of Time, P.O. Box 164, Pleasantville, N.Y. 10570, USA.)

July 1–7, **Ninth International Congress of Biochemistry.** (The Secretariat, 9th International Congress of Biochemistry, c/o Svenska Kemistsamfundet, Wenner-Gren Centre, 6tr S-113 46 Stockholm, Sweden.)

July 2–3, **Neutron Activation Analysis Symposium.** (Mr Peter Duggan, Symposium Secretary, Neutron Division, Marconi-Elliott Avionic Systems Limited, Elstree Way, Borehamwood, Herts.)

July 2–6, **The Sun in the Service of Mankind.** (Congrès International The Sun in the Service of Mankind, 28, Rue de la Source, Paris-XVIe, France.)

July 2–6, **Second AIC Congress "Colour 73".** (Professor W. D. Wright, Applied Optics Section, Imperial College, London SW7 2BZ.)

July 3–5, **Conference on Scanning Electron Microscopy Systems and Applications.** (The Institute of Physics, 47, Belgrave Square, London SW1X 8QX.)

July 3–6, **Molecular Motions in Liquids.** (Dr C. Troyannowsky, Secrétaire Général Société de Chimie Physique, 10, Rue Vauquelin, 75, Paris.)

July 4–5, **Anglo-French Symposium—Freeze Etching.** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford.)

July 4–6, **Fourth Canadian Wood Chemistry Symposium.** (Professor R. H. Marchessault, Department of Chemistry, Université de Montréal, C.P. 6128, Montreal 101, Quebec.)

July 5–6, **A Symposium on Genetic and Structural Aspects of Virus Genomes.** (The Meeting Assistant, Society for General Microbiology, Harvest House, 62, London Road, Reading RG1 5AS, Berks.)

July 6, **Symposium—X-ray Microanalysis of Biological Material in the Electron Microscope.** (The Administrator, Royal Microscopical Society, Clarendon House, Cornmarket Street, Oxford.)

July 8–14, **Twenty Years of Health Education: Evaluation and Forecast for the Years Ahead.** (Secretariat, VIIIth International Conference on Health Education, 20, Rue Greuze, 75 Paris 16e, France.)

July 9, **The Structure of Biological Molecules.** (Dr Lars-Johan Norrby, Structure of Biological Molecules, Department of Inorganic and Physical Chemistry, University of Stockholm, Roslagsvaegen 128, S-104 05 Stockholm, Sweden.)

July 9–20, **The Physics of Quantum Electronics.** (Professor Stephen F. Jacob and Marlan O'Scullly, Optical Sciences Center, The University of Arizona, Tucson, Arizona.)

July 10–12, **Conference on Video and Data Recording.** (The Information Officer, 8–9 Bedford Square, London WC1B 3RG.)

July 10–13, **Synthesis in Organic Chemistry.** (Dr John F. Gibson, The Chemistry

Society, Burlington House, London W1V 0BN.)

July 10–13, **The Fourth International Colloquium on Gasdynamics of Explosions and Reactive Systems.** (International Academy of Astronautics of the International Astronautical Federation, 250 Rue Saint-Jacques, 75 Paris, France.)

July 11, **Biochemistry and Treatment of Depression.** (Dr J. F. Cavalla, Society for Drug Research, John Wyeth & Brother Ltd., Huntercombe Lane South, Taplow, Maidenhead, Berks.)

July 11–13, **The Hundred and Seventy-second Meeting of the Genetical Society.** (Professor D. A. Hopwood, John Innes Institute, Colney Lane, Norwich.)

July 11–13, **European Nutrition Conference.** (Dr E. M. Widdowson, Dunn Nutritional Laboratory, Milton Road, Cambridge CB4 1XJ.)

July 11–13, **British Society for the History of Science—Summer Meeting.** (BSHS Office, 47 Belgrave Square, London SW1X 8QX.)

July 12, **The Annual General Meeting of the British Society.** (BSHS Office, 47 Belgrave Square, London SW1X 8QX.)

July 15–20, **Second International Conference on Culture Collections.** (Dr Antonio Fernando Pestana de Castro, Palácio das Convenções, Parque Anhembi 02012, São Paulo, SP, Brazil.)

July 15–20, **Symposium: Biotransformations and Fermentations.** (John M. Cassady, Ph.D., Chairman, ASP Publicity Committee, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana.)

July 16–19, **Ninth International Shock Tube Symposium.** (Symposium Secretary, Department of Aeronautics and Astronautics, Stanford University, Stanford, California.)

July 16–27, **Capri, Italy.** (Dr E. R. Pike, Royal Radar Establishment, Malvern, Worcs, U.K.)

July 16–28, **The Second International Conference on Permafrost in Yakutsk, Siberia.** (Dr Troy Péwé, Chairman, U.S. Planning Committee, National Research Council, 2101 Construction Avenue, N.W., Washington, D.C.)

July 17–20, **Conference on Pollution Criteria for Estuaries.** (Dr P. R. Helliwell, Department of Civil Engineering, The University, Southampton, SO9 5NH.)

July 17–20, **Biochemistry of Inositol Lipids Control of Fatty Acid Synthesis and Related Metabolism.** (Dr D. N. Brindley, Department of Biochemistry, University of Nottingham Medical School, Nottingham NG7 2RD.)

July 17–20, **Association for the Study of Animal Behaviour.** (Dr A. W. Ewing, Department of Zoology, West Mains Road, Edinburgh EH9 3JT.)

July 22-27, **Symposium on Man-Machine Communication for Scientific Data Handling.** (Secretary of the Task Group, c/o Mr M. K. Ward, International Conferences Office, National Research Council of Canada, Ottawa K1A 0R6, Canada.)

July 23-28, **The Fourth International Conference on the Global Impacts of Applied Microbiology.** (Dr Joao S. Furtado, Secretary-General GI AM IV, R. Gabriel dos Santos, 443, São Paulo, SP, Brazil.)

July 24-August 8, **1973 ESRO Summer School on European Manned Space Missions.** (European Space Research Organization, 114 Avenue Charles de Gaulle, 92-Neuilly-sur-Seine.)

July 31, **Protein Synthesis and Muscle Growth.** (Mr Claude Cruse, Business Manager, American Society of Animal Science, 425 Illinois Building, 113 North Neil Street, Champaign, Illinois.)

Reports and Publications

not included in the *Monthly Books Supplement*

Great Britain and Ireland

Biochemical Systematics, Vol. 1, No. 1, January 1973. Published quarterly. Pp. 1-78. Subscription rates: for libraries, university departments, government laboratories, industrial and other multiple reader institutions £12: \$30. Private individuals whose departmental libraries subscribe may obtain the journal for their personal use at a reduced rate of £5: \$12. (Oxford and New York: Pergamon Press, 1973.) [123]

London School of Hygiene and Tropical Medicine. Report on the work of the School, 1971/1972. Pp. 134. (London: London School of Hygiene and Tropical Medicine, 1973.) [123]

Potato Marketing Board, Sutton Bridge Experimental Station Annual Review—1972. Pp. 47. (London: Potato Marketing Board, 50 Hans Crescent, 1973.) [123]

The Birds from Your Window. Pp. 31. (Sandy, Bedfordshire: The Royal Society for the Protection of Birds, The Lodge, 1973.) *Gratts*. [123]

Mental Health Research Fund. Annual Report and Accounts 1972. Pp. 30. (London: Mental Health Research Fund, 1973.) [123]

Building Research Establishment Digest No. 151: Soakaways. Pp. 4. (London: HMSO, 1973.) 5p. [133]

National Radiological Protection Board. NRPB-R4: Dose Rates and Depth-Dose Distributions for Beta Particles Emitted by Commercially Available ⁹⁰Sr/⁹⁰Y, ²⁰⁴Tl, ¹⁴⁷Pm and ⁶³Ni Sources. By T. M. Francis and R. Scymour. Pp. 9. (Harwell, Didcot: National Radiological Protection Board, 1972.) [143]

Department of the Environment. Children at Play. (Design Bulletin 27.) Pp. 110. (London: HMSO, 1973.) 80p net. [143]

The Council of Engineering Institutions. Annual Report for 1972. Pp. 43. (London: The Council of Engineering Institutions, 2 Little Smith Street, 1973.) [143]

Countryside Commission. Landscape Agreements. Pp. 15. (London: Countryside Commission, 1973.) [153]

University of London. University College Annual Report, 1971/1972. Pp. 148. (London: University College London, 1973.) [163]

Other Countries

Australian Journal of Zoology, Supplementary Series, No. 19: The Primary Types of the Australian Eumastacidae (Orthoptera: Eumastacoidea). By K. H. L. Key. Pp. 40. (East Melbourne: Editorial and Publications Section, CSIRO, 372 Albert Street, 1973.) [123]

Report of the Council for Tobacco Research—U.S.A., Inc., for 1972. Pp. 103. (New York: The Council for Tobacco Research—U.S.A., Inc., 110 East 59th Street, 1973.) [123]

Australia: Commonwealth Scientific and Industrial Research Organization. The Division of Soils—Biennial Report on Progress, 1970/1971. Pp. 77. (East Melbourne: CSIRO, 1973.) [123]

Records of the Australian Museum. Vol. 28, No. 11: Earthworms (Megascopidae: Oligochaeta) from Mount Kosciusko, Australia. By B. G. M. Jamieson. Pp. 215-252. Vol. 28, No. 12: *Typton australis* SP. NOV., a New Pontoninid Shrimp from the Great Barrier Reef, Australia. By A. J. Bruce. Pp. 253-263. Vol. 28, No. 13: A New Species of *Notichus* (Homoptera: Fulgoroidea, Delphacidae) from Lord Howe Island. By R. G. Fennah. Pp. 265-267. Vol. 28, No. 14: Galatheaidea (Crustacea, Decapoda, Anomira) Collected by the F.I.S. Endeavour. By Janet Haig. Pp. 269-289. (Sydney: The Australian Museum, 1973.) [123]

Stockholm International Peace Research Institute. Report of Activities 1971/1972. Pp. 28. (Stockholm: SIPRI, Sveavägen 166, 1973.) [123]

US Department of the Interior: Geological Survey. Water-Supply Paper 2091: Quality of Surface Waters of the United States, 1968. Part 1: North Atlantic Slope Basins. Pp. x+373. (Washington, DC: Government Printing Office, 1972.) \$1.75. [123]

European Organization for Nuclear Research. CERN. CERN 73-1: Numerical Computation of Field Distribution and Frequency in the Lower Passbands of a Symmetrical Periodic Structure. Part 1: Theory. By M. Bell and G. Dome. Pp. 134. CERN 73-3: Visual Aids to Relativistic Kinematics. By G. C. Wick. Pp. 27. (Geneva: CERN, 1973.) [123]

Canada: Department of Energy, Mines and Resources. Paper 72-19: Description of Carboniferous and Permian Stratigraphic Sections, Northern Yukon Territory and Northwestern District of Mackenzie. By E. W. Bamber. Pp. v+161. (Ottawa: Information Canada, 1972.) \$2. [133]

Fisheries Research Board of Canada. Technical Report No. 361: Alimentary Tract Morphology of Selected North Atlantic Fishes in Relation to Food Habits. By A. V. Tyler. Pp. 21. Technical Report

No. 362: An Odometer for Use with Irish Moss Rakes. By D. J. Scarratt and S. M. Polar. Pp. 7. (St. Andrews, NB: Fisheries Research Board of Canada, Biological Station, 1972 and 1973.) [133]

Bulletin of the Institute of Jamaica, Science Series, No. 15, Part 1: The Botanic Garden, Liguanea (with a revision of *Horius eastensis*). By Dulcie Powell. Pp. 94. (Kingston, Jamaica: The Institute of Jamaica, 1972.) \$2. [143]

Indian Forest Records (New Series), Vol. 1, No. 3: Logging Terminology. Compiled by M. N. Asthana and D. N. Bhatia. Pp. 85-97. (Delhi: Manager of Publications, 1972.) Re.00.70. [143]

Sick Fund of the General Federation of Labour in Israel. Kupat-Holim Year Book, Vol. 2, 1972. Pp. 276. (Tel Aviv: The Family Physician, P.O.B. 16250, 1973.) [153]

International Council of Scientific Unions: Scientific Committee on Problems of the Environment. Scope Report No. 2: Man-Made Lakes as Modified Ecosystems. Pp. 78. (Paris: International Council of Scientific Unions, 1972.) [163]

United States Department of the Interior: Geological Survey. Professional Paper 742: Characteristics of Estuarine Sediments of the United States. By David W. Folger. Pp. vi+94. (Washington, DC: Government Printing Office, 1972.) \$1.25. [163]

Republique du Burundi. Institut des Sciences Agronomiques du Burundi. Rapports Techniques pour les Exercices 1963, 1964 et 1965 et Annexes. Pp. 147. (Bruxelles: Administration Generale de la Cooperation au Developpement de Belgique, 1972.) [163]

National Museums of Canada. Publications in Biological Oceanography. No. 4: The Caprellidae (Crustacea, Amphipoda) of Atlantic and Arctic Canada. By Diana R. Laubitz. Pp. 82. No. 5: Station Lists and New Distributional Records of Littoral Marine Invertebrates of the Canadian Atlantic and New England Regions. By E. L. Bonsfield and Diana R. Laubitz. Pp. 61. Publications in Zoology. No. 6: Ice-Worms (Oligochaeta: Enchytraeidae) from Western British Columbia. By Michael J. Tynen. Pp. 11. No. 7: Type Specimens of Mammals in the National Museum of Natural Sciences, Ottawa. By Philip M. Youngman. Pp. 7. Mercury Series. Archaeological Survey of Canada. Paper No. 5: NbV-1: An Historic Fishing Camp in Old Crow Flats, Northern Yukon Territory. By Richard E. Morlan. Pp. 44. Canadian Centre for Folk Culture Studies. Paper No. 5: Why Faith Healing? By Michael Owen Jones. Pp. 52. History Division. Paper No. 1: The Twenties in Western Canada—Papers of the Western Canadian Studies Conference, March 1972. Pp. 259. (Ottawa: National Museums of Canada, 1972.) [193]

Republique du Burundi: Ministère de l'Agriculture et de l'Elevage. Institut des Sciences Agronomiques du Burundi. Carte des Sols et de la Végétation du Burundi. 1. Planchette Muramvya. Notice Explicative de la Carte des Sols. Par R. Frankart et G. Sottiaux. Pp. 84. (Bruxelles: l'Administration Generale de la Cooperation au Developpement de Belgique et du Fonds Europeen de Developpement de la Communaute Economique Europeenne, 1972.) [193]

Regional Research Laboratory, Jorhat. Annual Report 1970/1971. Pp. 71. (Jorhat: Regional Research Laboratory, 1973.) [193]

Smithsonian Contributions to Zoology, No. 131: Two New Caridean Shrimps, One Representing a New Family, from Marine Pools on Ascension Island (Crustacea: Decapoda: Natantia). By Fenner A. Chace, Jr., and Raymond B. Manning. Pp. 18. (Washington, DC: Smithsonian Institution Press, 1972.) 30 cents. [193]

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